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EXPERIMENTS IN FIELD TECHNIC IN ROD ROW TESTS[1]

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INTRODUCTION

Present field practices in Farm Crops breeding and variety testing show a lack of uniformity in methods of work. This is partly because some investigators have larger plots of land at their disposal than others. The principal reason for the lack of a standard practice, however, is the lack of sufficient experimental evidence as to the reliability of different methods.

The close relation between laboratory methods and reliability of experimental results is accepted without question by workers in such exact sciences as chemistry and physics. While it is not possible to develop as exact practices in field work, owing to the many uncontrolled environmental factors, there is a possibility of standardization.

In order to accomplish such an end, it is essential that workers situated in various sections of the country make a study of the reliability of their present field technic.

In a consideration of this question there are several methods of attack. Some of these are the use of check plots, the value of replications, the size and shape of plots, methods of correcting the error of any plot due to soil heterogeneity, and the effects of competition between adjacent rows or plots.

In recent years there has been a tendency to use the row method in small-grain breeding and variety testing. The present paper is the first of a series of studies in field practices in Minnesota. The experimental data are of two sorts: (1) A study of the effects of competition between small grains planted in rod rows when spaced at a distance of 1 foot apart. (2) The value of replications for rod row tests.

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PREVIOUS STUDIES IN FIELD METHODS

Before the development of the row method, a series of attempts were made to develop a standard practice for field plot variety tests. Regarding many of the points mentioned in the earlier papers, there is as yet insufficient experimental evidence.

Smith (*13*) [1] in discussing corn variety tests mentioned the disadvantages of growing a short variety beside a taller one. The probability that some varieties have stronger foraging powers than others is given as a further reason for discarding border rows. Plots five rows wide are recommended.

Cory (*2*) presents the plan of using row plantings to check the results of field plot experiments. The plan outlined was the use of two rows 12 inches apart and 50 links long. From result of these studies the author concluded that the row results were more reliable as an indication of yielding ability than the results obtained from plot tests.

The work of Marek is cited by Wheeler (*16*) as showing the possible errors due to greater growth on the edges of plots. The plan of removing a certain border part of each plot before obtaining yields is recommended.

Piper and Stevenson (*10*) recommend certain practices for variety testing with the hope of standardizing methods. These recommendations are based on experimental studies of earlier investigators. Papers by Thorne (*15*) and Carleton (*1*) are especially mentioned as being valuable contributions to the idea of standardization. For corn variety work they adopted the method as outlined by Smith (*13*) with the addition of two to five replications for each variety and check plots every third or fourth plot. For small-grain testing the use of one-fortieth to one-tenth-acre plots in size with two to five replications and checks every third plot is recommended if one decides to use the plot method. For row tests a length of at least a rod with checks every fifth row and ten replications for each test is offered as a standard practice.

Mercer and Hall (*5*) reported a careful series of experimental tests in which they compared the relative reliability of results obtained from different-sized plots and gave results showing the reduction in error due to plot replication. The authors decided that one-fortieth acre plots were large enough for all practical purposes and recommended five replications for each variety under test. The conclusions of Lyon (*4*) in reference to plot size and replications are in substantial agreement with those of Mercer and Hall. Conclusive evidence is given to show that it is not possible to establish a schedule of relative yields for a series of plots even after several years' comparison.

Montgomery (*7*) presented an extensive study of the experimental error in nursery work with wheat for the characters nitrogen content

[1] Reference is made by number (italic) to "Literature cited," pp. 418–419.

and yield. He shows the value of a determination of the experimental error by the use of check plots. This error may then be used as a means of deciding which strains to eliminate. After determining the experimental error the rule given is

> to double the error and subtract this sum from the highest variant. The remainder after the subtraction represents the figure below which all strains could be discarded without danger of discarding a high strain

for the character studied. This conclusion refers only to the seasonal conditions under which the test is made.

From a standpoint of a study of nitrogen content Montgomery (7) states that single plants should be replicated 40 or more times, 16-foot rows 5 to 10 times, and blocks 5.5 feet square 8 to 16 times. From a yield standpoint the author finds blocks 5.5 feet square repeated 8 to 10 times to be as accurate as 16-foot rows repeated 15 to 20 times. This he believes to be due to the competition between adjacent rows, although no accurate data on this question are presented.

In a previous paper Montgomery (*6*) shows the effects of competition on the yield of different varieties when grown in the same row and planted alternately in the row. Under such conditions some varieties seem to be much more vigorous than others.

Increasing the length of the row or size of block is said by the author (7) to be a means of securing accuracy. An excellent size for rows was found to be 2 to 4 rods in length and blocks 5 by 16 feet in area.

Montgomery believes the bulk method of planting in nursery trials to be more closely correlated with field results than when the nursery plots are planted in such a manner that each plant has individual room for development. Data given for 11 varieties of oats tend to substantiate this belief.

Von Rümker, Leidner, and Alexandrowitsch (*12*) followed the practice in trials of pure lines of wheat for yield of making the distance between rows of the same pure line 13 cm. (5.1187 inches) and between the plots of any two pure lines 24 cm. (9.448 inches) to obviate any influence one pure line might exert on another growing in an adjacent row. No data are presented of the reasons which led them to adopt the method.

A recent paper of Pritchard (*11*) gives a report of a study made in 1912 in sugar-beet breeding investigations. Two characters, total sugar production per row and percentage of sugar, were used in the study. These were the basis for a discussion of the value of check plots and the comparative effect of repeated plantings upon the experimental error. The author concludes that check rows as a standard for the comparison of individual progeny rows are more efficient than the use of the means of the progeny rows as a standard for comparison. Check rows 32 inches from the progeny rows varied less than checks at a distance of 64, 96, 128, and 160 inches. Check rows at a distance of 64 to 160 inches seemed

to be about equally reliable as a comparison. In this work the employment of a check for every other row was said not to be sufficient to offset the variability due to soil irregularities; however, by means of 10 replications and alternate check rows the experimental error was reduced about 50 per cent. Reduction of the standard deviation was most rapid for the first 7 replications.

Numerous papers by Harris have called attention to the importance of the experimental error in variety testing. The more recent one (*3*) gives a method of determining the reliability of a field for experimental plot work. If irregularities in the field are large enough to affect areas larger than single plots, adjoining plots will be similarly affected. By the use of the correlation coefficient the correlation between the yield of the small plots and the yield of various groups of adjacent plots is determined. The more homogeneous the field the lower will be the correlation coefficient.

Surface and Pearl (*14*) have given a method of correcting plot yield which is partially based on Harris's correlation method. The calculated yield of any particular plot is determined by the contingency method. If this calculated yield is above or below the mean yield of the field, it is supposed that the soil of the plot is above or below the average of the field in productivity. The amount that the calculated yield is above or below the mean yield is then subtracted or added to the actual yield of the plot in question. The reliability of this method was then tested by the correlation plan as given by Harris. This test showed a considerable decrease in the heterogeneity of the field for the calculated yields as compared with the actual yields. The authors then show how this method may be used in variety tests.

MATERIAL AND METHODS OF WORK

In 1915 it was decided to make a test of the rod row as a preliminary method of determining performance. The method of handling the rows is about the same as is used at Cornell University in the Plant Breeding Department, although for the purposes of our work some modifications have been made.[1]

For our work 18-foot rows are used, spaced at a distance of 1 foot apart. At harvest 1 foot is discarded from each end of each row, thus leaving 16-foot rows. The use of the same length of row for wheat (*Triticum* spp.), oats (*Avena sativa*), and barley (*Hordeum vulgare*), increases the work of computing the yields per acre. The same length of row, however, for the various small grains allows a comparison of yield variability.

The rows were planted at the usual rate per acre for Minnesota, or 16.8 gm. per row for wheat, 15 gm. for oats, and 18.1 gm. for barley.

[1] Due acknowledgment is made to Dr. H. H. Love, of Cornell University, for descriptions of their row plans.

The work in 1915 was of a preliminary nature and does not necessitate an extensive discussion. It is only of interest as indicating the reason for the methods used in extensive tests made in 1916. In 1915 oat and wheat rows replicated nine times were grown for comparison with yields obtained in duplicated one-one hundred and tenth-acre plots. Correlation coefficients were calculated, giving the comparison between yields of 18 varieties of wheat and 21 varieties of oats. The coefficients are as follows:

Wheat..... $r = +0.593 \pm 0.123$
Oats..... $r = +0.200 \pm 0.141$

The same plot and series was used for these tests so that environmental conditions are relatively uniform. The fact that no closer relation in yield was obtained may be partially explained by the unreliability of the results as obtained in the duplicated one one-hundred-and-tenth-acre plot tests. (See following paragraph.) There is also the possibility of a greater competitive effect between adjacent rows of oats than for wheat.

A comparison of the relative reliability of single one one-hundred-and-tenth-acre plots and single rows of Bluestem wheat and Ligowa oats is given as a reason for further studies of the row method. The coefficient of variability is used as a measure of comparative reliability of the results of these tests (Table I).

TABLE I.—*Coefficients of variability for yield of wheat and oats as tested in one one-hundred-and-tenth-acre plots and rod rows, 1915*

Variety.	Type of plot.	Coefficient of variability.
Haynes Bluestem wheat.....	Single rod row.....	13.73±0.76
Do.....	Single one one-hundred-and-tenth-acre plot.	14.43±2.16
Ligowa oats.....	Single rod row.....	18.87±1.02
Do.....	Single one one-hundred-and-tenth-acre plot.	10.74±1.29

These tests indicate that a single row is as reliable as a single one-one hundred and tenth-acre plot for Haynes Bluestem wheat, although for oats the plot test appears to be more reliable. The result of this test, together with experiments of other workers, has led to the tentative adoption of one-fortieth-acre plots at University Farm. Further studies will determine the number of replications necessary for the plot work. The rod row test appears very promising, although further studies seem necessary, particularly as to competition between adjacent rows and number of necessary replications.

The main part of the experimental results here reported consist of studies of competition between adjacent rows of different varieties and

strains of wheat, oats, and barley and the decrease in variability due to replications.

The data obtained in 1916 is from two sources, namely, from rod row variety tests made by the Farm Crops Section and from rod row tests made in the Plant Breeding Nursery. The fields used for the work are at a considerable distance apart and have been handled in a different manner. In the presentation of results the tests will be spoken of as "Farm Crops test" and "Plant Breeding test," respectively.

In a considerable measure the uniformity of any particular field may be due to methods of handling. In the Farm Crops variety-test work the following rotations are used:

1. Oats, clover, corn.
2. Barley, hay, wheat, corn.

In the three-year rotation for the oats variety work there are two intervening crops. For the barley and wheat tests there is only one bulk crop intervening between the varietal tests.

In the Plant Breeding Nursery the following 7-year rotation is used: Rye, 10 pounds of clover in spring, clover hay, small-grain nursery, ear-to-row corn, field peas for seed, soybean variety test, and small-grain nursery.

In the 1916 test, plots of three rows, each replicated three times, were used in the Plant Breeding Nursery if sufficient seed was available and replicated five times for each variety reported in the Farm Crops test. Check plots of a standard variety were used every third to fourth plot. No attempt was made to correct for yield by the use of check plots. They were used, however, in a determination of the variability of the land and the value of replications.

It is realized that a single season's test does not furnish sufficient data upon which to draw a final conclusion as to a standard practice. The close relation between the development of reliable methods and the value of field tests, however, would seem to warrant the publication of results now available.[1]

COMPETITION BETWEEN ADJACENT ROWS

As each strain or variety tested was grown in at least one plot consisting of three rows of 16 feet in length, we may consider that each plot consists of one central row and two border rows which will be considered, respectively, as the right and left border rows. Each border row therefore grows near the same variety, the central row, on the one hand, and also beside some other variety or strain. In our work we have compared the yields of the border rows with each other, in relation to the height and yield of adjacent rows.

[1] Assistance in the computations was given by R. W. Garber and O. A. Haenert, student assistants.

CORRELATIONS FOR HEIGHT COMPETITION

The effects of the height of adjacent rows on the yield of the border rows may be first considered. The method of determining such effect is as follows: The yield of the border rows in bushels per acre was computed and the yield of the right border compared with that of the left. If the left border yielded the most, the result is given as a positive, or plus (+) yield, the difference being expressed in bushels per acre. If the right border yielded the most, the yield difference is a negative, or minus (−) result.

In somewhat similar manner the height of adjacent rows was compared. If the row of the variety or strain near the left border row was taller than the variety near the right border row, the result is given as a positive, or plus (+) difference, in inches; if the row near the right border was taller, the result is given as a negative, or minus (−) difference, in inches.

It should be noted that with the plant breeding barley work the height was taken in centimeters.

Correlation tables are then made in which the comparative yield of the border rows of the same variety is correlated with the comparative height of adjacent rows. If there is an effect on yield of border rows of the same variety owing to the height of adjacent rows, a negative coefficient should be obtained. A representative table for barley is given (Table II).

TABLE II.—*Correlation between the differences in yield between the border rows of a variety of barley and the differences in height (in centimeters) between the neighboring border rows on the left and right, respectively. Barley Plant-Breeding Nursery, 1916*

[Left greater = +. Right greater = −.]

Height, difference classes, adjacent border rows.	Yield, difference classes, border rows of same variety.													
	−9	−7.5	−6	−4.5	−3	−1.5	0	+1.5	+3	+4.5	+6	+7.5	+9	
−25	....	.....	....	.....	....	.....	1	.....	....	1	....	.....	....	2
−20	....	.....	....	.....	....	.....	1	.....	1	.....	....	1	....	3
−15	....	.....	....	.....	....	.....	.....	1	....	1	....	1	....	3
−10	....	.....	....	.....	....	1	.....	1	2	1	....	1	....	6
− 5	....	.....	....	.....	1	1	1	1	2	1	....	.....	1	8
0	....	.....	1	1	2	1	3	.....	3	2	....	.....	....	13
+ 5	2	.....	....	.....	2	3	.....	1	1	1	....	.....	....	10
+10	1	1	....	1	....	.....	1	.....	....	.....	....	.....	....	4
+15	....	.....	....	.....	....	1	.....	.....	1	.....	....	.....	....	2
+20	....	.....	....	.....	1	.....	.....	1	....	.....	....	.....	....	2
+25	....	.....	1	.....	1	.....	.....	.....	....	.....	....	.....	....	2
+30	....	.....	....	1	1	.....	.....	.....	....	.....	....	.....	....	2
.....	3	1	2	3	8	7	7	5	10	7	0	3	1	57

$r = -0.519 \pm 0.065$.

The correlation coefficients obtained for the various crops are given in Table III.

TABLE III.—*Correlations between the differences in yield between the border rows of a variety and the differences in height between neighboring border rows of unlike varieties of cereals*

Cereal.	Designation of test.	Number of tests.	Correlation coefficient.
Barley	Plant Breeding Nursery	57	−0.519±0.065
Do.	Farm Crops variety test	48	−.073±.097
Oats	Plant Breeding Nursery	218	−.111±.045
Do.	Farm Crops variety test	121	+.155±.060
Spring wheat	Plant Breeding Nursery	193	+.060±.048
Do.	Farm Cropse variety test	50	+.101±.094
Winter wheat	Plant Breeding Nursery	183	−.092±.049
Do.	Farm Crops variety test	38	−.337±.097

In the results in Table III as given for barley (Plant Breeding Nursery) each test is an average of three replications as the various varieties and strains are grown in the same order for each replication. In the winter-wheat results (Farm Crops Section) each test is an average of five plot results. With the exception of barley in the Plant Breeding Nursery and winter wheat in Farm Crops work, each test represents a single plot result.

In a consideration of these results certain environmental conditions are of interest. Until nearly the end of the crop season the barley and winter wheat in the Plant Breeding test stood up comparatively well. The oats and spring wheat stood up well until after heading and then nearly all oat plots lodged very badly, the spring wheat lodging considerably. A serious epidemic of black stemrust (*Puccinia graminis tritici* E. and H.), together with a very hot period shortly after heading, also caused serious reductions of yield in the Plant Breeding spring-wheat plots. In the Farm Crops work all cereals (with occasional exceptions) stood up well.

The correlation coefficients for the Plant Breeding work show considerable effect, owing to the height of adjacent rows on the border rows of the same variety in the barley tests and much smaller effects in the oats and winter-wheat tests.

In the Farm Crops test the correlation for the winter wheat is -0.337 ± 0.097. The odds against an occurrence of a deviation as large as 3.5 times the probable error is 1 to 82. There is only a slight indication of the effect of height in the barley tests, which is not great enough to be significant, and none for the oats or spring-wheat tests.

CORRELATIONS FOR YIELD COMPETITION

The effects of yield of adjacent rows on the border rows may be considered in a somewhat like manner as in the effects of height. The

results are obtained in a similar way as for the effects of height. Table IV is given as an illustration of the method used. This result shows considerable effect due to yield of adjacent rows on the border rows of the same variety.

TABLE IV.—*Correlation between the differences in yield of the border rows of a variety and the differences in yield between the adjacent rows on the left and right, respectively. Barley Plant Breeding Nursery, 1916*

[Left greater=+. Right greater=−]

Yield, difference classes, adjacent border rows.	Yield, difference classes, border rows of same variety.													
	−9	−7.5	−6	−4.5	−3	−1.5	0	+1.5	+3	+4.5	+6	+7.5	+9	
−21	....	.....	1	.....	....	.....	....	.....	....	.....	....	....	....	1
−18	....	.....	....	.....	....	.....	....	.....	....	.....	....	....	1	1
−15	....	.....	....	.....	....	.....	....	.....	1	.....	....	....	....	1
−12	....	.....	....	.....	....	.....	....	.....	....	.....	....	....	....	0
− 9	....	.....	....	.....	1	1	1	.....	2	3	....	....	....	8
− 6	....	.....	....	.....	....	.....	....	.....	2	2	....	1	....	5
− 3	....	.....	....	.....	....	.....	1	1	4	.....	....	1	....	7
0	....	.....	....	2	....	1	2	2	1	.....	....	....	1	9
+ 3	1	1	....	.....	3	1	....	1	1	3	....	....	....	11
+ 6	1	.....	....	.....	1	1	1	2	....	.....	....	....	....	6
+ 9	1	.....	1	.....	1	1	....	.....	....	.....	....	....	....	4
+12	....	.....	....	.....	1	.....	1	.....	....	1	....	....	....	3
+15	....	.....	....	.....	....	.....	....	.....	....	.....	....	....	....	0
+18	....	.....	....	1	....	.....	....	.....	....	.....	....	....	....	1
	3	1	2	3	7	5	6	6	11	9	0	2	2	57

$r = -0.394 \pm 0.075$

The correlation coefficients as obtained for the various crops are given in Table V.

TABLE V.—*Correlations between the differences in yield between the border rows of a variety and the differences in yield between adjacent rows of unlike varieties of cereals*

Cereal.	Designation of test.	Number of tests.	Correlation coefficients.
Barley	Plant Breeding Nursery	57	−0.394±0.075
Do.	Farm Crops variety test	48	− .040± .097
Oats	Plant Breeding Nursery	218	+ .100± .045
Do.	Farm Crops variety test	121	+ .017± .061
Spring wheat	Plant Breeding Nursery	193	+ .007± .049
Do.	Farm Crops variety test	50	− .290± .087
Winter wheat	Plant Breeding Nursery	183	+ .082± .050
Do.	Farm Crops variety test	38	+ .315± .099

These results show less effect of competition due to the yield of adjacent rows than in the case of the height of adjacent rows. The coefficient obtained in the Plant Breeding results for the barley test was

-0.394 ± 0.075. This is nearly six times the probable error, and thus it is statistically significant. The possibility that height and yield of adjacent rows was one and the same thing is dependent upon the question as to the relation of height and yield for the various crops. The correlation between height and yield for the 57 varieties of barley is $+0.214 \pm 0.085$.

The coefficient as obtained for spring wheat, Farm Crops test, shows some competition between adjacent rows due to yield.

Perhaps the most striking correlation coefficient is that for winter wheat, which is $+0.315 \pm 0.099$. As has already been mentioned, minus (−) coefficients show competitive effect. Positive (+) coefficients might be explained as due to soil heterogeneity.

ACTUAL EFFECT OF HEIGHT OF ADJACENT ROWS ON BORDER ROWS OF SAME VARIETY

The correlation coefficients as calculated show whether there is any competition between rod rows of small grains when the rows are spaced at a distance of 1 foot apart. The results as computed show that when varieties and strains of small grains are grown in rod rows there is an effect due to the height of adjacent rows in the case of barley, oats, and winter wheat in the Plant Breeding test and no effect for the spring-wheat test. The correlation coefficients for the oat and winter-wheat results show only a small effect, the coefficients being a little less than three and two times the probable errors, respectively.

In the Plant Breeding barley test the effect is a large one, and it seems worth while to demonstrate the actual effect of certain height differences. In considering these results certain facts will be repeated. The height of each plot was measured in centimeters, and there was no appreciable difference in height between the border and central rows in each plot. If there is any effect on border rows of the same variety due to differences in the height of adjacent rows one might expect, on the average, that the border row which grew near a short variety might yield more than a row of the same sort which happened to be near a tall variety. If this is so, the result is considered as a positive, or plus (+) difference. The average increase, in bushels per acre, is also given. Negative results are preceded by a minus (−) sign. The results are given in 3-cm. classes for the difference in height of the neighboring plot (Table VI).

The results in Table VI show that the actual effect on rows of the same variety due to the height of adjacent rows is of the utmost importance in rod row tests under conditions such as obtained for the barley in the Plant Breeding tests.

TABLE VI.—*Results on yield of border rows of the same variety of barley due to differences in height of adjacent rows*

Average differences in height of adjacent rows.	Number of tests.	Number positive.	Number negative.	Average differences in yield of border rows (in bushels. per acre).
Cm.				
30	2	2	0	4.1
27	2	2	0	4.8
24	2	2	0	1.7
21	3	2	1	3.3
18	2	2	0	1.5
15	4	3	1	1.9
12	7	7	0	4.9
9	8	6	2	3.5
6	10	8	2	2.1
3	16	11	5	− .1
0	9	7	2	+ .7

Similar results as presented for oats (Plant Breeding test) show a substantial agreement between the correlation coefficients calculated and results as obtained for the actual test of effects of certain specific height differences. These results are given in Table VII.

TABLE VII.—*Results on yield of border rows of the same variety of oats due to differences in the height of adjacent rows*

Average differences in height of adjacent rows.	Number of tests.	Number positive.	Number negative.	Average differences in yield of border rows (in bushels. per acre).
Inches.				
12	2	2	0	9.8
10	2	1	1	1.2
9	2	1	1	−4.8
8	2	0	2	−3.5
7	4	2	2	3.3
6	10	6	4	2.0
5	13	9	4	1.7
4	29	16	13	2.6
3	34	21	13	3.4
2	57	26	31	− .7
1	67	31	36	−1.8
0	53	23	30	− .3

The total minus (−) results for 1 to 12 inches difference in height are obtained by multiplying the average results by the number of tests and obtaining $\Sigma = 177.1$. The total plus (+) results are obtained in similar manner. $\Sigma = 268.3$.

VARIABILITY OF BORDER ROWS, CENTRAL ROWS, AND THREE-ROW PLOTS OF CHECK PLOTS OF HAYNES BLUESTEM WHEAT, LIGOWA OATS, MANCHURIA BARLEY, AND TURKEY WINTER WHEAT

As a further study of the effects of competition between adjacent rows of small grains, the variability for the yield of the border rows and the central rows, as well as a comparison of the value of central rows and 3-row plots is important. The results are presented in Table VIII. The number of tests, the place of test and the respective means, standard deviations, and coefficients of variability are given.

TABLE VIII.—*Comparison of variability of border rows, central rows, and 3-row plots of check plots of Haynes Bluestem wheat (Minnesota 169); Ligowa oats (Minnesota 281); Manchuria barley (Minnesota 105); and Turkey winter wheat (Minnesota 529)*

Plot or row.	Designation of test.	Number of tests.	Mean.	Standard deviation.	Coefficient of variability.
Wheat:					
Central rows	Plant Breeding Nursery.	51	11.7 ±0.190	2.01±0.134	17.25±1.187
Left border rows	do	51	11.1 ± .199	2.11± 141	19.08±1.325
Right border rows	do	51	12.0 ± .221	2.34± .156	19.44±1.350
3-row plots	do	51	11.6 ± .157	1.66± .111	14.30± .974
Central rows	Farm Crops variety test.	15	20.6 ± .261	1.50± .185	7.28± .896
Left border rows	do	15	21.1 ± .317	1.82± .224	8.63±1.063
Right border rows	do	15	20.1 ± .277	1.59± .196	7.91± .974
3-row plots	do	15	20.7 ± .240	1.38± .170	6.67± .821
Oats:					
Central rows	Plant Breeding Nursery.	59	58.3 ± .598	6.81± .423	11.68± .732
Left border rows	do	59	55.5 ± .761	8.67± .538	15.61± .989
Right border rows	do	59	57.7 ± .731	8.33± .517	14.43± .914
3-row plots	do	59	57.2 ± .587	6.68± .415	11.69± .733
Central rows	Farm Crops variety test.	20	61.2 ± .849	5.63± .600	9.20± .981
Left border rows	do	20	61.8 ± .908	6.02± .642	9.74±1.039
Right border rows	do	20	61.9 ±1.106	7.33± .782	11.84±1.281
3-row plots	do	20	61.6 ± .885	5.87± .626	9.53±1.016
Barley:					
Central rows	Plant Breeding Nursery.	9	36.2 ± .949	4.22± .672	11.66±1.872
Left border rows	do	9	36.6 ±1.857	8.26±1.313	22.57±3.696
Right border rows	do	9	38.0 ± .710	3.16± .502	8.32±1.323
3-row plots	do	9	37.0 ± .924	4.11± .653	11.11±1.784
Central rows	Farm Crops variety test.	15	32.0 ± .381	1.90± .234	5.94± .731
Left border rows	do	15	31.0 ± .441	2.53± .312	8.16±1.005
Right border rows	do	15	30.5 ± .509	2.92± .360	9.57±1.178
3-row plots	do	15	31.3 ± .298	1.71± .211	5.46± .672
Winter wheat:					
Central rows	Plant Breeding Nursery.	72	27.5 ± .370	4.65± .261	16.91± .979
Left border rows	do	72	27.4 ± .419	5.27± .296	19.21±1.119
Right border rows	do	72	26.0 ± .406	5.11± .287	19.65±1.149
3-row plots	do	72	27.0 ± .327	4.12± .232	15.26± .875
Central rows	Farm Crops variety test.	41	29.1 ± .504	4.78± .356	16.43±1.256
Left border rows	do	41	28.9 ± .529	5.02± .374	17.37±1.332
Right border rows	do	41	27.6 ± .473	4.49± .334	16.27±1.244
3-row plots	do	41	28.4 ± .403	3.83± .285	13.49±1.023

As has already been mentioned, the Farm Crops test is on a different series and under a different system of rotation than the Plant Breeding work. Furthermore, the check plots for the Farm Crops work occurred every third or fourth plot, while the Plant Breeding checks were placed every fifth plot. These differences may partially explain the lack of agreement in the coefficients of variability as determined for the tests.

The data as given allows for two main comparisons, both of which are of importance in a discussion of rod rows as a means of determining yield.

These are the relative variability for yield of the border and central rows and the relative variability of central rows and 3-row plot tests.

It is entirely possible in a limited number of tests that if there is a competitive effect between adjacent rows that in some cases this might tend to lower the variability of the test. For example, if a plot happened to be situated in a particularly favorable soil location, the rows would be expected to yield more than the average for that particular variety. If, in addition, the border rows of this plot grew near a much taller variety or beside a stronger foraging variety, the effect of competition would be to lower the yield and thus reduce the calculated coefficient of variability for the variety in question. For this reason the coefficient of variability for the yield of the central rows is compared with the most variable border row test. Results of such comparisons are given in Table IX. The probable error of the difference between coefficients of variability being calculated by the following formula, the error of a difference is equal to the square root of the sum of the squares of the quantities entering into the difference (*8*).

TABLE IX.—*Comparison of the difference between the coefficient of variability of the border and central rows*

Cereal.	Designation of test.	Difference between the coefficient of variability of the border and central rows.	Difference ÷ probable error.
Wheat (Minnesota 169).....	Plant Breeding Nursery....	2.09±1.788	1.2
Do.....	Farm Crops variety test	1.35±1.390	.9
Oats (Minnesota 281).....	Plant Breeding Nursery.....	3.93±1.230	3.2
Do.....	Farm Crops variety test	2.64±1.613	1.6
Barley (Minnesota 105).....	Plant Breeding Nursery.....	10.91±4.208	2.6
Do.....	Farm Crops variety test	3.63±1.386	2.6
Winter wheat (Minnesota 529)	Plant Breeding Nursery.....	2.74±1.509	1.9
Do.....	Farm Crops variety test	.94±1.838	.5

The results show no significant difference for the spring- and winter-wheat tests between the coefficients of variability of border and central rows. The chances against an occurrence of a deviation as large as 1.9 times the probable error as obtained in the Plant Breeding winter-wheat test is 4.00 to 1. For barley the odds against an occurrence of a deviation as large as 2.6 times the probable error, as obtained for both Farm Crops and Plant Breeding tests, is 11.58 to 1 (*9*). The odds against an occurrence as great as 1.6 and 3.2 times the probable error as obtained for the Farm Crops and Plant Breeding tests for oats are, respectively, 2.57 to 1 and 31.36 to 1. It seems fair to conclude that border rows are more variable than central rows for barley and oat tests and that the differences in variability of border and central rows of spring- and winter-wheat tests here reported are of no great importance.

The comparison between the coefficients of variability for the 3-row plots and central rows is a means of determining the value of plot size. The results would seem to be more significant in the case of spring and

winter wheat where there is apparently little competition between adjacent rows than for the barley and oat tests.

Table X gives the comparison for the coefficients of variability for yield of the central rows and of the 3-row plots.

TABLE X.—*Comparison of the difference between the coefficients of variability of central rows and 3-row plots*

Cereal.	Designation of test.	Difference between coefficient of variability of central rows and 3-row plots.	Difference ÷ probable error.
Wheat (Minnesota 169).....	Plant Breeding Nursery....	2.95±1.535	1.9
Do.....	Farm Crops variety test	.61±1.215	.5
Oats (Minnesota 281).....	Plant Breeding Nursery....	−.01±1.036	.....
Do.....	Farm Crops variety test	−.33±1.412	.....
Barley (Minnesota 105).....	Plant Breeding Nursery....	.55±2.596	.2
Do.....	Farm Crops variety test	.48± .993	.5
Winter wheat (Minnesota 529).	Plant Breeding Nursery....	1.65±1.313	1.3
Do.....	Farm Crops variety test.....	2.94±1.699	1.7

The results as reported for the comparison of central rows and 3-row plots (Table X) are in substantial agreement with the results for a comparison of central and border rows (Table IX). For the oats and barley the central rows are no more variable for yield than the 3-row plots. For these crops the border rows had a significantly greater coefficient of variability for yield than was obtained for the central rows.

In the winter- and spring-wheat tests there was little indication of competitive effect of adjacent rows, with the exception of the winter-wheat Farm Crops test, as determined by the coefficient of variability of the border and central rows. Table X shows that with winter and spring wheat the use of all three rows for each check plot in computing yield was somewhat more accurate than the use of central rows. The difference between the coefficient of variability for yield for the central rows and 3-row plots is 1.9, 0.5, 1.3, and 1.7 times the probable error. The probability of the occurrence of deviations as large as 1.7 and 1.9 times the probable error are, respectively, 2.98 to 1 and 4.00 to 1. The slight reduction in error in winter and spring wheat due to the use of all three rows of the 3-row plots does not appear to be of sufficient size to warrant the work involved in harvesting, threshing, and computing the border-row yields.

VALUE OF REPLICATIONS

The two methods of overcoming soil variations of any particular season's test are the determination of the sort and size of plot to use and systematic plot replications. Plots consisting of 3-rod rows each were used for our test. Each row was then harvested separately and the yield per acre computed. The value of replications was determined

for the central rows and for the 3-row plots, respectively. For these tests the standard varieties used in the check plots in the spring-wheat, oats, barley, and winter-wheat tests have been used.

The probable errors are often very large, owing to the small number of plots or rows in these tests in which many replications are studied. As the tests for the Farm Crops and Plant Breeding work are made in widely separated fields, except in the winter-wheat tests, it would seem that the conclusions for the particular crop year in question would be fairly sound.

The method of replication used for the work is as follows: If there were 50 check plots in a particular test and 2 replications were to be studied, an average of plot 1 and plot 26, plot 2 and plot 27, etc., was taken. Similarly for 3 replications and 51 plots the average of plot 1, plot 18, and plot 35, etc. was taken.

The value of replications in Haynes Bluestem wheat is given in Table XI.

TABLE XI.—*Value of replication of central rows and 3-row plots of Haynes Bluestem wheat (Minnesota 169) used as a check in the Farm Crops and Plant-Breeding tests*

Designation of test.	Method of test.	Number of tests.	Mean.	Standard deviation.	Coefficient of variability.
Farm Crops variety test..	Single central rows.....	15	20.6±0.261	1.50±0.185	7.28±0.896
Do.....	2 replications.....	7	20.6± .191	.75± .135	3.64± .656
Do.....	3 replications.....	5	20.6± .323	1.07± .228	5.19±1.107
Do.....	4 replications.....	3	20.7± .370	.95± .262	4.59±1.264
Do.....	6 replications.....	2	20.8± .167	.35± .118	1.68± .566
Do.....	3-row plots.....	15	20.7± .240	1.38± .170	6.67± .821
Do.....	2 replications.....	7	20.7± .250	.98± .177	4.73± .853
Do.....	3 replications.....	5	20.7± .314	1.04± .222	5.02±1.071
Do.....	4 replications.....	3	20.7± .136	.35± .096	1.69± .465
Do.....	6 replications.....	2	20.7± .119	.25± .084	1.21± .408
Plant Breeding Nursery..	Single central rows.....	51	11.7± .100	2.01± .134	17.25±1.187
Do.....	2 replications.....	25	11.6± .174	1.29± .123	11.08±1.067
Do.....	3 replications.....	17	11.6± .239	1.46± .169	12.60±1.487
Do.....	4 replications.....	12	11.8± .193	.99± .136	8.37±1.164
Do.....	6 replications.....	8	11.6± .165	.69± .116	5.94±1.002
Do.....	9 replications.....	5	11.4± .241	.80± .171	7.02±1.497
Do.....	3-row plots.....	51	11.6± .157	1.66± .111	14.30± .974
Do.....	2 replications.....	25	11.6± .158	1.17± .112	10.12± .975
Do.....	3 replications.....	17	11.5± .128	.78± .090	6.76± .782
Do.....	4 replications.....	12	11.8± .162	.83± .114	7.06± .972
Do.....	6 replications.....	8	11.8± .157	.66± .111	5.62± .948
Do.....	9 replications.....	5	11.6± .148	.49± .104	4.22± .900

For the Farm Crops test of Minnesota 169 wheat there were only 15 plots available. For this reason the Farm Crops results for the value of replications are less reliable than the Plant Breeding tests, where 51 plots were grown. In the Farm Crops work for the central-row replication studies the use of any number of replications from two to four seems about equally accurate. Six replications, as compared with two, gave a difference in the standard deviation of 0.40±0.179. This is slightly more than 2 times the probable error.

For the test of replications in the 3-row plots two or three replications appear to be about equally reliable. Four replications are much more accurate than three, the difference in the standard deviation of four

and three replications being 0.69±0.242. This is 2.8 times the probable error. The chances of a deviation as large as 2.8 times the probable error are 1 to 15.95. Six replications appear to be no more accurate than four replications.

In comparing the test as made by the use of central rows and 3-row plots it would seem that for one, two, or three replications the central rows were about as accurate as the 3-row plots. Four replications in 3-row plots are apparently as reliable as six replications for central rows.

For the Plant Breeding test two or three replications of central rows appear to be about equally reliable. Four replications seem to be about as valuable as either six or nine. The difference in the standard deviation as given for three or four replications is 0.47±0.217. This is of possible value.

For the test of replications in the 3-row plots there is in general a slight progressive reduction in variability as the number of replications increases from one to nine. The largest reductions are from one to two and from two to three replications. The progress reductions between three and nine replications are only very small.

In like manner the value of replications has been studied for the oat check plots. In a consideration of the results for central rows and 3-row plots it is of interest to again call attention to the greater effect of competition in the Plant Breeding test between adjacent rows of oats than for the wheat tests.

Data for a comparison of the value of replications in the oat tests are presented in Table XII.

TABLE XII.—*Value of replication of central rows and 3-row plots of Ligowa oats (Minnesota 281) used as a check in the Farm Crops and Plant Breeding tests*

Designation of test.	Method of test.	Number of tests.	Mean.	Standard deviation.	Coefficient of variability.
Farm Crops variety test..	Single central rows.....	20	61.2±0.849	5.63±0.600	9.20±0.981
Do.....	2 replications.....	10	61.2± .970	4.55± .686	7.43±1.121
Do.....	3 replications.....	6	61.6±1.013	3.68± .717	5.97±1.162
Do.....	4 replications.....	5	61.2±1.020	3.38± .721	5.52±1.177
Do.....	6 replications.....	3	61.6± .732	1.88± .518	3.05± .840
Do.....	9 replications.....	2	61.5± .076	.16± .054	.26± .088
Do.....	3-row plots.....	20	61.6± .885	5.87± .626	9.53±1.016
Do.....	2 replications.....	10	61.6± .932	4.37± .659	7.09±1.069
Do.....	3 replications.....	6	62.2±1.019	3.70± .720	5.95±1.159
Do.....	4 replications.....	5	61.6± .953	3.16± .674	5.13±1.094
Do.....	6 replications.....	3	62.3± .534	1.37± .377	2.20± .606
Do.....	9 replications.....	2	62.3± .706	1.48± .499	2.38± .803
Plant Breeding Nursery..	Single central rows.....	59	58.3± .598	6.81± .423	11.68± .782
Do.....	2 replications.....	29	58.9± .587	4.69± .415	7.97± .706
Do.....	3 replications.....	19	58.7± .484	3.13± .342	5.33± .583
Do.....	4 replications.....	14	58.9± .633	3.51± .447	5.96± .760
Do.....	6 replications.....	9	58.3± .645	2.87± .456	4.92± .782
Do.....	9 replications.....	6	58.0± .388	1.41± .275	2.43± .473
Do.....	3-row plots.....	59	57.2± .587	6.68± .415	11.69± .733
Do.....	2 replications.....	29	57.7± .510	4.07± .360	7.05± .624
Do.....	3 replications.....	19	57.2± .532	3.44± .376	6.02± .659
Do.....	4 replications.....	14	57.6± .508	2.82± .359	4.89± .623
Do.....	6 replications.....	9	57.0± .551	2.45± .390	4.30± .684
Do.....	9 replications.....	6	56.9± .240	.87± .169	1.53± .298

Twenty plots were used for the Farm Crops test and 59 for the Plant Breeding work. There appears to be very little difference in variability as determined by the Farm Crops or Plant Breeding results, the Plant Breeding coefficients of variability for the respective replications being only slightly larger than those which were obtained for the Farm Crops test.

In the Farm Crops test there is a large reduction in the standard deviation for two replications as compared with a single replication. A slight reduction in variability for three as compared with two replications is apparent. Three or four replications appear to be about equally reliable. There is a large reduction in the standard deviation due to six replications as compared with three. For the central row tests the difference in the standard deviation for yield of six replications as compared with three is 1.80 ± 0.884. This is slightly more than two times the probable error.

The comparison of 3-row plots and central rows shows little difference for any number of replications except nine, and such differences as are obtained for this number of replications are in favor of the use of central rows as compared with 3-row plots.

For the Plant Breeding test there is little difference between the use of 3-row plots and central rows for any number of replications below nine. There is an apparent advantage in favor of the use of 3-row plots for nine replications. As this result is exactly the reverse of the Farm Crops result for nine tests, it would indicate that these results are chance fluctuations.

For the central rows three replications appear to be as reliable as any number below nine. There is some advantage in the 3-row plot tests for six as compared with three replications. This is, however, of doubtful significance, the difference in the standard deviations being 0.99 ± 0.541. Nine replications appear to be as reliable as nine replications for the Farm Crops work. It is of interest to note that three replications reduce the error about 50 per cent as compared with a single test.

The results for replications in barley are given in Table XIII.

TABLE XIII.—*Value of replication of central rows and 3-row plots of Manchuria barley (Minnesota 105) used as a check in the Farm Crops and Plant Breeding tests*

Designation of test.	Method of test.	Number of tests.	Mean.	Standard deviation.	Coefficient of variability.
Farm Crops variety test..	Single central rows.....	15	32.0±0.331	1.90±0.234	5.94±0.731
Do.....	2 replications.....	7	32.0± .428	1.68± .303	5.25± .946
Do.....	3 replications.....	5	32.0± .317	1.05± .224	3.28± .670
Do.....	4 replications.....	3	32.0± .386	.99± .273	3.09± .851
Do.....	6 replications.....	2	32.0± .191	.40± .135	1.25± .422
Do.....	3-row plots.....	15	31.3± .298	1.71± .211	5.46± .672
Do.....	2 replications.....	7	31.2± .247	1.36± .245	4.36± .786
Do.....	3 replications.....	5	31.3± .284	.94± .200	3.01± .642
Do.....	4 replications.....	3	31.3± .167	.43± .118	1.37± .377
Do.....	6 replications.....	2	31.3± .095	.20± .067	.64± .216
Plant Breeding Nursery..	Single central rows.....	9	36.2± .949	4.22± .672	11.66±1.872
Do.....	2 replications.....	4	36.6± .907	2.69± .642	7.35±1.753
Do.....	3 replications.....	3	36.2±1.050	2.70± .743	7.46±2.054
Do.....	3-row plots.....	9	37.0± .924	4.11± .653	11.11±1.784
Do.....	2 replications.....	4	37.6± .853	2.53± .603	6.73±1.605
Do.....	3 replications.....	3	36.9±1.102	2.83± .779	7.67±2.112

For the tests of replications with barley there were 15 plots available in the Farm Crops test and 9 in the Plant Breeding test.

The Farm Crops work showed a lower variability in all cases than for the Plant Breeding results. For the Farm Crops test two replications proved to be only slightly more valuable than a single test. In the central row tests three and four replications seem to be about equally reliable, while six replications reduce the error about 50 per cent as compared with three replications. The use of 3-row plots gave a lower error as determined by the coefficient of variability and the standard deviation than the use of central rows alone.

For the Plant Breeding tests central rows or 3-row plots show about the same standard deviation. Two or three replications reduce the error nearly 40 per cent.

The results for replication studies in winter wheat are given in Table XIV. In considering these results it should be noted that both the Plant Breeding and Farm Crops tests were made on the same soil series.

TABLE XIV.—*Value of replications of central rows, 3-row plots, and border rows of Turkey winter wheat (Minnesota 529) used as a check in the Farm Crops and Plant-Breeding tests*

Designation of test.	Method of test.	Number of tests.	Mean.	Standard deviation.	Coefficient of variability.
Farm Crops variety test..	Single central rows.....	41	29.1±0.504	4.78±0.356	16.43±1.256
Do.....	2 replications.....	20	29.6± .593	3.93± .419	13.28±1.441
Do.....	3 replications.....	13	29.1± .406	2.17± .287	7.46± .987
Do.....	4 replications.....	10	29.4± .469	2.20± .332	7.48±1.128
Do.....	6 replications.....	6	28.5± .666	2.42± .471	8.49±1.653
Do.....	9 replications.....	4	28.8± .762	2.26± .539	7.93±1.891
Do.....	3-row plots.....	41	28.4± .403	3.83± .285	13.49±1.023
Do.....	2 replications.....	20	28.4± .338	2.24± .239	7.89± .841
Do.....	3 replications.....	13	28.5± .393	2.10± .278	7.37± .975
Do.....	4 replications.....	10	28.4± .397	1.86± .281	6.55± .988
Do.....	6 replications.....	6	28.1± .774	2.18± .547	7.76±1.511
Do.....	9 replications.....	4	28.0± .661	1.96± .467	7.00±1.669
Plant Breeding Nursery..	Single central rows.....	72	27.5± .370	4.65± .261	16.91± .979
Do.....	2 replications.....	36	27.6± .335	2.98± .237	10.80± .868
Do.....	3 replications.....	24	27.4± .346	2.51± .244	9.16± .892
Do.....	4 replications.....	18	27.6± .238	1.49± .169	5.43± .610
Do.....	6 replications.....	12	27.3± .391	2.01± .277	7.36±1.013
Do.....	12 replications.....	6	27.3± .333	1.21± .236	4.43± .863
Do.....	3-row plots.....	72	27.0± .327	4.12± .232	15.26± .875
Do.....	2 replications.....	36	27.1± .310	2.76± .219	10.18± .817
Do.....	3 replications.....	24	27.2± .304	2.21± .215	8.16± .794
Do.....	4 replications.....	18	27.1± .234	1.47± .165	5.42± .609
Do.....	6 replications.....	12	27.1± .323	1.66± .229	6.13± .844
Do.....	12 replications.....	6	27.2± .333	1.21± .236	4.45± .866

There is very little difference for the Farm Crop tests between the variability as determined by central rows or 3-row plots with the exception of one or two replications, which are lower in the 3-row plot than for the central row tests. Three replications appear to be about as reliable as any larger number.

The Plant Breeding test shows no significant difference in the variability as determined by the use of 3-row plots or central rows for any number of replications studied. Four replications for both central rows

and 3-row plots seem significantly better than three, although there is some doubt about the accuracy of this conclusion when one notes that four replications give a lower variability than six. Twelve replications as compared with three show a difference of 3.71 ± 1.041.

SUMMARY OF RESULTS

(1) In a study of competition between rod rows of small grains grown 1 foot apart there was some effect on the yield of border rows of the same variety due to height of adjacent rows of barley, winter wheat, and in one of two tests an indication of such effect in oats. The results were variable in different plots, such variation being due possibly to the environmental conditions. There was no apparent effect of height of adjacent rows on the yield of border rows of the same variety in spring wheat.

(2) The yield of adjacent rows appeared to be of some importance in barley tests and in the Farm Crops spring-wheat tests. Correlations obtained for other tests indicated considerable soil heterogeneity.

(3) The effects of the height of adjacent rows for the barley Plant-Breeding tests was unquestionable. The effects of the height of adjacent rows were sufficient to often cause differences of 4 or 5 bushels per acre in the yield of border rows of the same variety of barley.

(4) The comparison of yield variability of border and central rows of check plots of barley, oats, spring and winter wheat was further evidence of the competitive effect of rod rows of small grains when grown 1 foot apart. In nearly all tests the border rows proved to be more variable in yield than the central rows.

(5) The check plots of oats, spring and winter wheat, and barley were used for a study of the value of replications in reducing error. In nearly all tests three replications as compared with a single plot reduced error by 25 to 50 per cent.

(*a*) In the spring-wheat Farm Crops tests for central rows four replications gave about the same coefficient of variability as nine replications for the Plant Breeding test. In the Farm Crops series six replications reduced the error by over 50 per cent as compared with three replications. The lack of agreement between the Plant Breeding and Farm Crops test may be explained by the serious black-stemrust epidemic which attacked the Plant Breeding Nursery.

(*b*) For the Farm Crops oat test six replications reduced the error by about 25 per cent as compared with three replications. For the Plant Breeding tests three replications were about as valuable as any higher number below nine.

(*c*) For the barley tests three replications seemed to be about as accurate as any number below six.

(*d*) For the winter-wheat results three or four replications reduced the error about 50 per cent and appeared to be about as accurate as any number below 12.

CONCLUSIONS

The studies here reported show the necessity of the determination of field methods of technic in Plant Breeding and Farm Crops plot tests.

Conclusive evidence is given which shows that in some cases there is considerable competition between rod rows of small grains when grown a distance of 1 foot apart. This has led to the adoption of three-row plots at University Farm. The results of a comparison of three-row plot yields with central rows show that central rows are as accurate a determination of yield as the use of all three rows.

In a study of replications for rod rows of small grains considerable variability was shown for the different tests. In general three or four replications seem to be about as accurate a method as the use of any number below nine. The indications are that 9 to 12 replications would reduce error due to soil heterogeneity to a minimum.

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HYDROCYANIC-ACID GAS AS A SOIL FUMIGANT

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INTRODUCTION

Though much has been written on soil fumigation, it can not be said that we have a satisfactory method for treatment against soil insects. Most of such work has been done in France with carbon disulphid against phylloxera, but no reliable data on soil work exist regarding the availability of hydrocyanic-acid gas, the most efficient fumigant known in orchard and house work; hence, the following study was made of the action of hydrocyanic-acid gas in the soil and its effect on germinating seeds and plants.

Any extensive treatment of the soil must be considered in relation to crops grown thereon, especially those occupying the ground for a period of one year or more. Operations possible while the ground is occupied by a crop are much to be preferred, although knowledge of the treatment of unplanted areas is very desirable. The experiments to be described in this paper were made largely with the idea of the treatment of occupied ground, for it follows as a matter of course that treatment which is safe for planted areas is also safe for bare land.

Two distinct lines of work are necessary for the study of this subject:

(a) Establishing a definite ratio between the minimum point of toxicity to insects and the maximum dosage which is safe to germinating seeds and plants.

(b) A study of the physical and chemical action of the gas in the soil; the rate and extent of diffusion and absorption by soil water; the adsorption of the gas by soil particles and its decomposition by certain soil constituents.

A. RELATION OF POINT OF TOXICITY OF INSECTS AND PLANTS

Many data have been accumulated by the various Experiment Stations, and particularly by the California Station, regarding the toxicity of hydrocyanic-acid gas to insects. Likewise there are many data (*1–8*) [1] available on the effects of the gas on plants, but practically all of them are based on house experiments where the actual concentration of the gas is not accurately known. Therefore it was necessary to make a few tests to establish a basis of comparison.

A method elaborated by Prof. C. W. Woodworth, of the California Station, in the investigation of the action of hydrocyanic-acid gas on the eggs of certain insects, makes use of the vapor-tension laws of gases.

[1] Reference is made by number (italic) to "Literature cited," p. 436.

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It was found that the concentration of hydrocyanic-acid vapor in the air over a simple aqueous solution of the gas quickly becomes constant if a relatively large volume of the solution were held in a small closed container. Furthermore, if the temperature is constant, it was shown that the concentration of the hydrocyanic-acid vapor in air under the above conditions was dependent on the concentration of the solution.

A solution of hydrocyanic-acid gas was made by dissolving 1 gm. of sodium cyanid in 1 liter of water and adding 15 c. c. of 10 per cent sulphuric acid. (This is actually an aqueous solution of hydrocyanic-acid gas in a sodium-sulphate solution, probably containing a slight excess of sulphuric acid.) The air in a closed container above a relatively large volume of such a solution contained approximately 0.3 per cent of hydrocyanic-acid vapor,[1] which is within the range of strength used for fumigation work.

If air were slowly aspirated through this solution, it also would contain approximately 0.3 per cent hydrocyanic-acid vapor. Other solutions were made up similar to the above, but of different concentrations, the amount of sodium cyanid being reduced one-half in each succeeding culture until the minimum point of toxicity was reached.

Houseflies (*Musca domestica*) were exposed to the following range of dilutions in closed test tubes (Table I):

TABLE I.—*Concentration of gas used on houseflies*

Sodium cyanid per liter.	Approximate concentration of gas.	Sodium cyanid per liter.	Approximate concentration of gas.
Gm.	*Per cent.*	*Gm.*	*Per cent.*
1.0	0.3000	0.0625	0.0187
.5	.1500	.0312	.0093
.25	.0750	.0156	.0046
.125	.0375		

[1] The method of calculating the amount of hydrocyanic-acid gas in air:

A standard sodium-cyanid solution is made up as above, containing 1 gm. of sodium cyanid per liter and 15 c. c. of 10 per cent sulphuric acid. Air is aspirated first through this solution, then through *N/100* iodin solution (containing a little sodium carbonate) until the latter is completely decolorized, the ratio being 36.5 c. c. of air (the mean of a number of experiments) = 1 c. c. of *N/100* iodin.

One equivalent of iodin is equal to two equivalents of HCN, according to the sense of the following equation:

$$HCN + I_2 = HI + ICN;\ \text{therefore}$$

1 c. c. *N/100* I = 0.0001351 gm. HCN

1 c. c. *N/100* I = 36.5 c. c. air

36.5 c. c. air = 0.0001351 gm. HCN

$$1 \text{ c. c. air} = \frac{0.0001351}{36.5} \text{ gm. HCN}$$

$$1{,}000 \text{ c. c. air} = \frac{0.0001351}{36.5} \times 1{,}000 = \frac{0.1351}{36.5} = 0.0037 \text{ gm. HCN in 1 liter of air}$$

1 liter air = 0.0037 gm. HCN

1 liter air = weighs 1.29 gm. at 0° C. and 760 mm.

Barometric pressure 755.6

Thermometer 20° C.

$$1{,}000 \text{ c. c.} \times \frac{755.6}{760} \times \frac{273}{293} = 937.2 \text{ c. c. air}$$

937.2 c. c. weighs 1.2099 gm.

$$\frac{0.0037}{1.2099} \times 100 = 0.306 \text{ per cent HCN in air.}$$

In the concentration of 0.0156 gm. of sodium cyanid per liter (equivalent to 0.0046 per cent of hydrocyanic-acid gas over the solution) many of the flies were dead at the end of 1½ hours; the others, being apparently normal, were freed. A second series of experiments with hydrocyanic-acid gas was made on the following insects: Phylloxera (*Phylloxera vastatrix*), tree hoppers (*Stictocephala festina*), leaf hopper (*Agallia* sp.), houseflies (*Musca domestica*), beetles (*Diabrotica soror*), with the results given in Table II.

TABLE II.—*Effect of hydrocyanic-acid gas on insects*

[Time of exposure one hour]

Sodium cyanid per liter.	Approximate concentration of gas above liquid.	Effect on insects experimented upon.			
		Phylloxera.	Leaf hoppers.	Houseflies.	Beetles.
Gm.	*Per cent.*				
1.0	0.3	Dead...	Dead...	Dead...	Dead.
.5	.15	...do....	...do....	...do....	Do.
.25	.075	...do....	...do....	...do....	Do.
.125	.0375	...do....	...do....	...do....	Do.
.0625	.0187	...do....	...do....	...do....	Alive.
.0312	.0093	...do....	...do....	...do....	Do.
.0156	.0046	...do....	...do....	...do....	Do.
.0078	.0023	...do....	...do....	10 per cent alive.	Do.
.0039	.0011	Alive...	Alive...	Alive...	Alive.

A concentration of approximately 0.0046 per cent hydrocyanic-acid gas in the atmosphere within the test tube was the minimum point of toxicity to flies in both experiments. For beetles a gas eight times as strong—that is, 0.037 per cent of hydrocyanic-acid gas—is necessary to cause death.

GERMINATION TESTS WITH HYDROCYANIC-ACID GAS SOLUTIONS[1]

Tests were made on alfalfa (*Medicago sativa*), turnip (*Brassica rapa*), corn (*Zea mays*), and lettuce (*Lactuca sativa*) seed to determine the effect of hydrocyanic-acid gas solutions on germination. Glass tumblers were used as containers for the solution, the seeds being placed in the glass on swings which just touched the surface of the water. The tumblers were sealed with paraffined paper held in place with a rubber band. The temperature of the room throughout the experiment ranged from 19.5° to 24° C. Fifteen dilutions of the hydrocyanic-acid gas solu-

[1] One point should be noted in this entire series of experiments—viz, the possibility of a very slight excess of sulphuric acid in the solution of hydrocyanic-acid gas. With these experiments it was the intention to use just enough sulphuric acid to combine with the sodium and free all the hydrocyanic-acid gas. In some cases all the acid might not have been neutralized and possibly would of itself cause some injury, or the sodium sulphate, one of the products resulting from the reaction of sulphuric acid with sodium cyanid, might also have some action on the plant.

tions were made, the amount of sodium cyanid being reduced one-half in each succeeding culture, No. 16 being a control of pure water. The first tumbler contained 2.3485 gm. of sodium cyanid to the liter, approximately a 1.15 per cent solution of hydrocyanic acid, or 0.704 per cent hydrocyanic-acid gas in the air above the liquid. The seeds were placed in the glasses and allowed to remain for 48 hours before the first reading was taken. At this time most of the unsprouted seeds were removed from the first 10 solutions and placed in swings similar to those above described, but in pure water. The record for these is found in Table III under the heading "Removed from solution." In the first column will be found the amount of sodium cyanid used in making the hydrocyanic-acid gas solutions; the second column gives the record of the seeds remaining in the solution; and the third column the record of the seeds removed after remaining 48 hours in the hydrocyanic-acid gas solution.

TABLE III.—*Summary of germination tests 11 days after planting*

Sodium cyanid in 1 liter.	Percentage of sprouted seeds—		Sodium cyanid in 1 liter.	Percentage of sprouted seeds—	
	Remaining in solution.	Removed from solution after 48 hours.		Remaining in solution.	Removed from solution after 48 hours.
Gm.	*Per cent.*	*Per cent.*	*Gm.*	*Per cent.*	*Per cent.*
2.3485	0.0	1.25	0.0091	98.7	90.00
1.1742	.0	13.00	.0045	92.0	66.00
.5871	25.0	27.30	.0022	97.7	.....
.2935	25.0	57.60	.0011	100.0	.....
.1467	55.1	80.60	.00056	98.2	.....
.0733	43.3	79.00	.00028	97.0	.....
.0366	76.1	98.00	.00014	99.0	.....
.0183	88.7	86.00	Water.	91.5	.....

In the record of the seeds removed from the hydrocyanic-acid gas solutions it will be seen that a very large percentage germinated except in the very strongest solutions. The germination record of those remaining in the original solution is much lower; thus showing that the seeds removed were only retarded in their germination by the action of the strong hydrocyanic-acid gas solution. In practical work, with hydrocyanic-acid gas in the soil, where no attempt would be made to hold the concentration of gas at a high point for a week or more, the dangerous stage might soon be past and we would have instead a stimulating effect. It will be noticed that the maximum acceleration came at different points in the two methods of treatment—0.0011 gm. of sodium cyanid per liter (equivalent to 0.00033 per cent of hydrocyanic-acid gas over the solution) when the seeds remained in the solution 11 days, and 0.0366 gm. of sodium cyanid per liter (equivalent to 0.0109 per cent of hydrocyanic-acid gas over the solution) for the seeds moistened for only 48 hours and then placed in pure water. The most striking

effect (not shown in Table III) was the more rapid growth of the seedlings and their deeper color in the dilution immediately adjacent to where the definite retarding action ceased. A diagram of the heights is shown in figure 1.

The maximum height of the alfalfa seedlings was reached at a dilution of 0.0022 gm. of sodium cyanid per liter (equivalent to 0.0006 per cent of hydrocyanic acid over the solution).

EFFECT OF HYDROCYANIC-ACID GAS ON CUTTINGS AND SEEDLINGS

1. PLANTS IN SOIL WATERED WITH HYDROCYANIC-ACID GAS SOLUTIONS

Seedlings of lettuce and turnip and six weeks' old turnips and beets were planted in tumblers containing soil, and watered with hydrocyanic-acid gas solutions, 1 ounce of the solution being used to the tumbler.

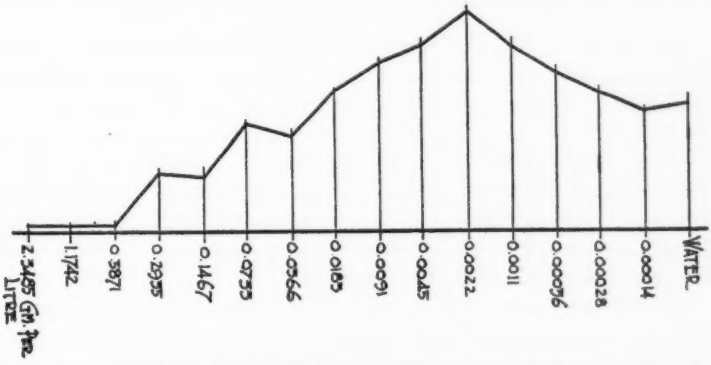

FIG. 1.—Diagram showing the relative heights of the alfalfa seedlings grown in hydrocyanic-acid gas solutions produced by adding sulphuric acid to sodium cyanid in solution.

The first solution contained 18.779 gm. of sodium cyanid per liter, with a proportionate amount of acid as in the germinating experiments, giving approximately a 5.6 per cent of hydrocyanic-acid gas over the solution, the amount of sodium cyanid being reduced one-half each time through a series of 12 graduations, the thirteenth culture being a control of pure water. One or more plants were placed in each tumbler and sealed with paraffined paper held in place with a rubber band. The covering paper was slit to the center, the plant inserted in the opening and the edges of the paper drawn together and sealed with melted paraffin close up to the stem of the plant. In this way the roots were exposed to the hydrocyanic-acid solution and to a sodium-sulphate solution, but the gas did not come in contact with the leaves.

All the plants in the first five containers were killed and only in the soil watered with the ninth solution which contained 0.0733 gm. of sodium cyanid per liter (equivalent to 0.0219 per cent of hydrocyanic acid over the solution) did the plants remain normal.

2. PLANTS WITH ROOTS IMMERSED IN AQUEOUS SOLUTIONS OF HYDROCYANIC-ACID GAS

Turnip and lettuce seedlings and three months' old turnip plants were removed from the soil in which grown and the roots were immersed in hydrocyanic-acid gas solutions and sealed in as above. In this experiment, as in the former, all the plants were killed down to and including dilution 1.1742 gm. of sodium cyanid per liter (equivalent to 0.3522 per cent of hydrocyanic-acid gas over the solution). The minimum point at which the plants were normal was very close to the first series; but there was this difference: In three dilutions, 0.0091 to 0.00225 gm. of sodium cyanid per liter (equivalent to 0.00273 to 0.000675 per cent of hydrocyanic-acid gas over the solutions) an acceleration in growth was noticed, while none was noted in the first series.

3. CUTTINGS IN AQUEOUS SOLUTION OF HYDROCYANIC-ACID GAS

The various solutions of hydrocyanic-acid gas were made up as before, and cuttings of chrysanthemum (*Chrysanthemum* sp.) and wandering jew (*Zebrina pendula*) were partially immersed in the solutions. The plants in this series were the most resistant of any of those tried. From Table IV it will be seen that only in solutions containing 9.394 gm. of sodium cyanid per liter (equivalent to 2.818 per cent of hydrocyanic-acid gas over the solution) were all of the cuttings killed, the point of normality being 0.0733 gm. of sodium cyanid per liter (equivalent to 0.0219 per cent of hydrocyanic acid gas over the solution).

The experiments on plants thus far have been with aqueous solutions of hydrocyanic-acid gas, in which some part of the plant was immersed. To make these experiments comparable with those on insects where the gas was used (instead of an aqueous solution of hydrocyanic-acid gas), it must be known whether or not the effect of hydrocyanic-acid gas on the leaves is comparable to the action of the gas solution on the roots.

4. PLANTS WITH LEAVES EXPOSED TO HYDROCYANIC-ACID GAS, WITH ROOTS PROTECTED

The ordinary-sized tumbler was partially filled with water, and inside the tumbler a whisky glass was placed containing ½ ounce of hydrocyanic-acid gas solution, the first one having 9.394 gm. of sodium cyanid per liter (equivalent to 2.818 per cent of hydrocyanic-acid gas over the solution) and being reduced one-half in each succeeding culture, as in previous experiments. Small seedling turnips were placed between the walls of the two glasses, with the roots extending into the pure water. Melted paraffin was then poured on the surface of the water, thus sealing in the plants and preventing the roots from coming in contact with the gas.

By comparing the results with those of plants in aqueous solutions of hydrocyanic-acid gas it will be seen that the action was similar. With the gas acting only upon the leaves, all were killed, up to and including

a dilution of 0.5871 gm. of sodium cyanid per liter (equivalent to 0.1761 per cent of hydrocyanic-acid gas over the solution), while in the aqueous solutions all were killed, up to and including a dilution of 1.1742 gm. of sodium cyanid per liter (equivalent to 0.352 per cent of hydrocyanic-acid gas over the solution). Stimulation was shown in both series at about the same dilution. In the columns of Table IV showing plants in aqueous solutions of hydrocyanic-acid gas and soil watered with hydrocyanic-acid gas solutions, a very close similarity will be seen in the effect on the plant, whether the roots are entirely immersed or the plant is grown in a soil moistened with hydrocyanic-acid gas solutions.

TABLE IV.—*Summary of effect of hydrocyanic-acid gas on plants*

Sodium cyanid per liter.	Soil watered with solutions of hydrocyanic-acid gas.	Roots in aqueous solutions of hydrocyanic-acid gas.	Cuttings in aqueous solutions of hydrocyanic-acid gas.	Leaves exposed to hydrocyanic-acid gas.
Gm.				
18.788	Dead.			
9.394	do.	Dead.	Dead.	Dead.
4.697	do.	do.	50 per cent shriveled. 50 per cent dead.	Do.
2.3485	do.	do.	Shriveled.	Do.
1.1742	do.	do.	Tips growing. Stems shriveled.	Do.
.5871	50 per cent shriveled. 50 per cent dead.	25 per cent alive. 75 per cent dead.	20 per cent alive. 80 per cent dead.	Do.
.2935	25 per cent normal. 75 per cent dead.	50 per cent normal. 50 per cent dead.	Tips growing. Stems wilty.	Partially dried.
.1467	50 per cent normal. 50 per cent dead.	88 per cent normal. 12 per cent dead.	75 per cent normal. 25 per cent dead.	Do.
.0733	Normal.	94 per cent normal. 6 per cent dead.	Normal.	Tender leaves dead. Dead.
.0366	do.	Normal.	do.	Acceleration.
.0183	do.	do.	do.	Do.
.0091	do.	20 per cent acceleration. 80 per cent normal.	do.	Normal.
.0045	do.	90 per cent normal. 10 per cent acceleration.	do.	Do.
.00225	do.	5 per cent acceleration. 95 per cent normal.	do.	Do.
Water.	do.	Normal.	do.	Do.

The minimum dosage for killing all the lettuce seedlings, the most susceptible plants experimented with, was, in the case of gas applied to the leaves only, a dilution 0.5871 gm. of sodium cyanid per liter (equivalent to 0.176 per cent of hydrocyanic acid gas over the solution), and at this concentration each of the plants tested showed a death rate of from 50 to 80 per cent when the solutions were applied to the roots. From this it will be seen that the gas alone is almost as toxic when applied to the leaves as the hydrocyanic-acid gas solution is when applied to the roots.

TABLE V.—*Minimum dosages toxic to insects experimented upon*[a]

Insect.	Sodium cyanid per liter.	Approximate concentration of gas.
	Gm.	*Per cent.*
Phylloxera	0.0078	0.0023
Houseflies	.0156	.0046
Beetles	.125	.0375

[a] Maximum dosage harmless to all plants experimented with both in the gaseous and solution form of the acid was 0.0366 gm. of sodium cyanid per liter, approximately 0.0109 per cent of hydrocyanic-acid gas over the solution.

These experiments indicate that a dosage toxic to flies and phylloxera—viz, 0.0156 gm. of sodium cyanid per liter (equivalent to 0.0046 per cent of hydrocyanic-acid gas over the solution)—would be safe for all the plants experimented upon; but for the beetles used so much stronger dosage is required that it would be extremely injurious, if not fatal, to every plant experimented upon. Before planted areas could be treated it would be necessary to determine experimentally the minimum dosage fatal to the insects present and compare that with plants growing thereon, besides considering reactions of the gas and the soil. There would probably be a considerable margin of safety on some soils between the killing strengths necessary for an insect, such as the phylloxera, and injury to more hardy plants, especially in the dormant season.

B. PHYSICAL-CHEMICAL ACTION OF HYDROCYANIC-ACID GAS IN THE SOIL

If hydrocyanic-acid gas is used as a soil fumigant, it will be necessary to fill the soil with gas, either from sodium cyanid placed in the soil and decomposed into hydrocyanic-acid gas, or by generating the gas at the surface and forcing it into the soil. Tubes sunk into the soil to various depths make it possible to draw out the gas for measurement. The concentrations thus determined, compared with the data given above on the toxicity of the gas to insects, would indicate the probabilities of the presence of a killing strength of hydrocyanic-acid gas in the soil.

For drawing gas out of the soil ¼-inch gas pipe cut into 36-inch lengths was used. The tubes may be sunk to any depth, up to 30 inches, at which a determination is desired. To operate, the tube is sunk into the ground a short piece of rubber tubing is drawn over the end projecting out of the

soil; into the open end of this tubing a rubber stopper (through which a short piece of glass tubing is inserted) is forced, effectually sealing the soil tube. The glass tubing in the stopper is the outlet through which the soil air is drawn. These tubes will be spoken of as "sampling tubes," in order to distinguish them from the "soil tube" used later on in the experiment for studying the diffusion of gas in the soil.

Experiments were begun in a clay-loam soil. In this the sampling tubes were sunk to a depth of 30 inches, and sodium cyanid in four different forms was introduced into the soil at the same depth.

1. Lump sodium cyanid was placed in the ground, and double the amount of sulphuric acid necessary to free the gas was poured on it, but the acid soaked into the ground before the sodium cyanid was dissolved.

2. An aqueous solution of sodium cyanid without acid was poured down one of the tubes and air drawn off from a second tube 1 foot distant from the first and at the same depth, the air being aspirated through 25 c. c. *N/100* iodin; no trace of hydrocyanic-acid gas was shown in several trials.

3. An aqueous solution of sodium cyanid was poured into a pipe as before, followed with the usual amount of acid necessary to release all the gas, likewise without recovering the gas by aspiration through an adjacent tube.

4. Various forms of apparatus for generating gas on the surface and forcing it into the soil were used, the first one being known as the "Babo generator," in which only enough pressure was generated to force the gas out of the generator. A modified form of this apparatus was then prepared, in which large quantities of gas were generated, with slightly more pressure, but with no apparent advantage. Another style of generator was devised by Prof. Woodworth, giving a pressure of over 100 pounds per square inch; but, on trial, results similar to those in the other experiments were obtained, there being no apparent advantage in generating gas in quantity or at a considerable pressure. These were the most promising forms of apparatus used in generating gas on the surface, but all were so unsatisfactory that attention was turned to generating gas in the soil.

The actual generation of gas in the soil must be delayed during the operation long enough to allow for the covering of the generator with soil to a depth sufficient to prevent any loss of gas. Provision must also be made to prevent the filling of the generator with soil or the loss of acid before the reaction is complete.

A pasteboard box, paraffined on the inside to resist the action of the acid, or a tin can with a tight-fitting lid, may be used for the generator. In order to delay the action of the acid on the sodium cyanid until the preparations are completed, we wrapped the cyanid in paper or placed it in pasteboard boxes and then dropped it into the acid solution. Table VI gives the result of an experiment in a dry clay-loam soil.

TABLE VI.—*Hydrocyanic-acid gas generated in a compact clay-loam soil*

Depth of generator.	Charge of sodium cyanid.	Distance of test from generator.	Time elapsing after fumigation.	Results.
Inches.	*Ounces.*	*Inches.*		
24	19	18	1 minute....	No trace of hydrocyanic-acid gas.
24	19	18	14 hours.....	Do.
24	19	6	.....do.....	Do.
24	19	Directly at generator.	.....do.....	0.48 per cent of hydrocyanic-acid gas.
24	19	.....do.....	60 hours.....	0.144 per cent of hydrocyanic-acid gas.

At the top of the hole where the generator had been inserted there was no odor of gas at any time during or after the generation. The only trace of hydrocyanic-acid gas, except directly at the generator, was just 6 inches from the generator, and even at this point it was much below the point of toxicity to insects. This experiment showed definitely that heavy soils are very impervious to this gas.

Work was now begun on sandy soil. A box 2 feet deep, 20 inches wide, and 2 feet 4 inches long was filled with moist sand, the generator being placed near the bottom and at one end of the box. Table VII gives the results.

TABLE VII.—*Hydrocyanic-acid gas generated in loose, sandy soil*

Depth of generator.	Charge of sodium.	Distance of test from generator.	Time elapsing after fumigating.	Results.
Inches.	*Ounces.*	*Inches.*		
20	12	18	1 minute....	0.12 per cent of hydrocyanic-acid gas.
20	12	18	16 hours.....	0.3 per cent of hydrocyanic-acid gas.
20	12	18	64 hours.....	Faint trace.

By comparing the first and second reading it will be seen that several hours are required for the gas to diffuse even a short distance in the soil. When the gas was first generated the odor was very noticeable near the generating box and also when the box was emptied after taking the last of the three readings. The gas, which is temporarily absorbed by the water, is given off again as the partial pressure in the soil atmosphere decreases.

The water in the soil takes up large quantities of gas and retains it in solution a long time, so that, even though the concentration of gas in any soil might not be great enough to be fatal to insects on a short exposure, yet with the longer periods of time which we seem to have in soil work, there is a possibility of a smaller dose being effective than would be the case where the gas escapes more quickly.

LABORATORY STUDY OF SOIL ABSORPTION

From the above experiment it was seen that the gas was either being absorbed by the soil moisture, adsorbed[1] by the soil particles, or that it reacted chemically with the soil constituents.

A series of experiments was therefore begun to determine the effect of water and of different soils on hydrocyanic-acid gas. Air from a jar containing 1.0 gm. of sodium cyanid and 15 c. c. of 10 per cent sulphuric acid in 1 liter of water was aspirated through 5 c. c. of *N/100* iodin until the latter was decolorized, this requiring 468 c. c. of air. To determine the amount of gas absorbed by water, air from the same solution of hydrocyanic-acid gas as above was aspirated through 50 c. c. of water and then into iodin until the latter was decolorized, the result being that 3,456 c. c. of air decolorized 5 c. c. of *N/100* iodin.

AIR ASPIRATED DIRECTLY INTO IODIN

It was found that 468 c. c. of air from the above solution decolorized 5 c. c. of *N/100* iodin when introduced directly, while it was necessary to use 3,456 c. c. of air from the same solution to decolorize an equal amount of iodin when aspirated first through 50 c. c. of water, showing that the latter absorbed the gas from 2,988 c. c. of air, equivalent, according to the above data, to 31 c. c. of *N/100* iodin.

To obtain an approximate idea of the amount of hydrocyanic-acid gas necessary to saturate the soil at a definite partial pressure of hydrocyanic-acid gas, air from the standard solution as above was aspirated through a tube containing 50 gm. of soil until no more gas was absorbed. At the first titration it required 2,740 c. c. of air when drawn through the soil to decolorize 5 c. c. of *N/100* iodin, while 540 c. c. direct from the hydrocyanic-acid gas solution gave the same result. The difference between the amounts aspirated through the soil and that directly from the gas solution gave the total amount of hydrocyanic-acid gas absorbed, or adsorbed, or chemically decomposed by the soil, which was equivalent to 26 c. c. of *N/100* iodin, in contrast with the equivalent of 31 c. c. of *N/100* iodin absorbed by a similar amount of water. Table VIII indicates the gradual approach to the point of saturation.

TABLE VIII.—*Saturation of soil with hydrocyanic-acid gas*

Character of soil.	Titration.	Control.	Difference.
	C. c.	*C. c.*	*C. c.*
Sandy loam	2,740	540	2,200
Do	880	510	370
Do	620	510	110
Do	580	510	70
Do	460	415	45
Total quantity of air required to saturate soil	.....	.....	2,795

[1] The term "adsorption" will be used to designate the concentration of two or more heterogeneous phases at their contiguous surfaces in contrast with the volume concentrations of these same substances. "Absorption" is the phenomenon of interpenetration of two or more phases until a certain concentration in the total volume is reached.

A large series of soils ranging from pure sand, and soils with a large percentage of gravel, to heavy adobe was then treated as before, sufficient air from the standard hydrocyanic-acid-gas solution being aspirated through 50 gm. of the different soils until enough hydrocyanic-acid gas came over to decolorize 5 c. c. of *N/100* iodin.

Table IX gives the results of the experiments with the soil samples used. The column headed "Titration" gives the quantity of air from the hydrocyanic-acid-gas solution that was aspirated through 50 gm. of soil and then through 5 c. c. of *N/100* iodin until the latter was decolorized. The "Control" column gives the quantity of air from the same hydrocyanic-acid-gas solution that was aspirated directly through a second 5 c. c. of *N/100* iodin until it was decolorized, the difference between these two figures being an index of the amount of hydrocyanic-acid gas retained by the soil. The sixth column is a calculation of the number of pounds of sodium cyanid required to furnish as much hydrocyanic-acid gas for 1 acre, 3 feet deep, as was found to be adsorbed or decomposed chemically by the experimental sample, this amount being based on the difference between the results given in the columns under "Titration" and "Control," plus the amount required to saturate the soil at a definite partial pressure of hydrocyanic-acid gas. The seventh column gives the amount of sodium cyanid which it is estimated will give a killing strength of hydrocyanic-acid gas in the pore space of the soil and should be added to the amount which is adsorbed or decomposed chemically by the soil. The pore space in most soils was figured at 35 per cent by allowing 1 ounce of sodium cyanid per 100 cubic feet, it would require 28.5 pounds of cyanid to fumigate this space. This gives the effective dose for 1 acre, the amount being given in the eighth column. The estimated cost for fumigating 1 acre to a depth of 3 feet is given in the last column.

TABLE IX.—*Amounts of sodium cyanid required on different soils* [a]

Sample.	Locality.	Character of soil.	Titration.	Control.	Quantity of sodium cyanid per acre neutralized by soil.	Estimated amount per acre to give killing strength.	Effective dose per 3 acre-feet.	Cost per acre.[b]
			C. c.	*C. c.*	*Pounds.*	*Pounds.*	*Pounds.*	
1	Orland	Gravelly	1,742	404	831	25	856	$231.52
2	do	do	2,450	650	673	25	698	188.46
3	do	do	1,260	438	458	25	483	130.41
4	Marysville	Sandy loam	3,868	913	789	28	807	217.89
5	Chico	Clay loam	5,063	379	3,000	30	3,030	818.10
6	do	do	4,990	620	1,720	30	1,750	472.50
7	Oroville	do	2,106	446	940	28	968	261.36
8	Sacramento	do	3,540	515	1,430	28	1,458	393.66
9	do	do	2,240	560	730	28	758	204.66
10	Berkeley	do	3,632	728	975	30	1,005	271.35
11	Stockton	do	4,115	515	1,700	28	1,728	466.56
12	do	do	2,740	540	990	27	1,017	274.56

[a] Three feet, or even more, is the depth necessary to fumigate soil for phylloxera. For most insects one-half of this depth would be sufficient.

[b] The cost is figured on a basis of 27 cents a pound for sodium cyanid.

These requirements show the wide range of absorptive power of different soils, varying approximately from 3,030 pounds of sodium cyanid for a clay loam to 483 pounds for a gravelly soil. The figures given of the cost for the lighter soils indicate that the use of sodium cyanid, while comparable with fumigation with carbon disulphid, is not within the range of economy except in small areas, even when the value of the gas as a nitrogenous fertilizer is considered. The fumigation of heavy soils is very much more expensive.

From the difficulties encountered while working with the generators it seemed necessary to study more carefully the rate of diffusion and absorption of hydrocyanic-acid gas through the various soil media and the effect of soil moisture on this movement. Five experiments were made covering different types of soil and varying amounts of soil moisture. After introducing the hydrocyanic-acid gas into the soil, tube readings were taken at intervals ranging from a few minutes to 120 hours.

1. Heavy clay soil—Readings taken following the introduction of the hydrocyanic-acid gas and 48 hours later.

2. Wet clay-loam soil containing considerable humus—Readings taken immediately after charging the tube.

3. Very sandy, beach soil, containing about 5 per cent of broken shell slightly moist—First reading taken immediately, the next 16 hours later.

4. Clean river sand washed and dried at a temperature of 200° C. to remove the soil, organic matter, and moisture—Readings taken immediately after charging with gas and 5 days later.

5. Sand washed and dried as above and afterwards saturated with water—Readings taken immediately.

For this purpose a copper soil testing tube 36 inches long and 2 inches in diameter was made. One end of the testing tube is closed with the exception of a ⅛-inch tube, the opposite end is closed with a rubber stopper through which is inserted a ⅛-inch glass tube; at intervals of 6 inches along the sides ⅛-inch tubes are inserted; to all of these small tubes a short piece of rubber hose is attached which may be closed with clamps. Air from a standard solution of hydrocyanic-acid gas was drawn in through the tube inserted in the stopper, by applying a vacuum at any of the tubes along the sides or at the opposite end of the testing tube, all the openings being closed except the ones where the air was admitted. Then by detaching the tube from the acid solution and drawing in pure air at the same point the amount of gas in that section of the tube could be measured, and also the amount lost could be calculated. On account of the small amounts of gas present in the different sections, qualitative tests only with silver nitrate ($AgNo_3$) was substituted for the iodin method. This overcomes the error from volatilization of

iodin caused by aspirating through it large quantities of air. Turbidity with silver nitrate was reached with about 120 c. c. of air from the standard hydrocyanic-acid gas solution when 450 c. c. of air at the same concentration of gas would neutralize 1 c. c. of *N/100* iodin.

These experiments, made in loose soil, emphasize the slow rate of diffusion of hydrocyanic-acid gas in the soil, which rate would necessarily be very much slower, and perhaps would be checked entirely in field conditions. Diffusion seems to be accomplished to a large extent by at least a partial saturation of the soil body through which it is moving (Table X). In experiment 1 the concentration of the gas seemed quite uniform throughout the tube, but in experiment 1a, after standing 48 hours, a decided decrease is shown in the last section of the tube, indicating that the outer part was not saturated to the same extent as the sections through which the air was drawn. Solubility of the gas in water is indicated in experiment 2 (wet soil), the outer end of the tube not having a trace of gas, while the concentration in the first three sections is very high. The chemical reaction or adsorption of the dry gas by the clay soil over a period of time is also shown in experiments 1 and 1a, where the quantity of air required for turbidity increases from 160 c. c. immediately after charging the tube to 1,200 c. c., 48 hours afterwards. The most striking difference in the retention of hydrocyanic-acid gas is shown between sand and clay soils. In experiment 1, 108 liters of air from the hydrocyanic-acid gas solution were aspirated through the tube and in experiment 3 only 66 liters of air from the hydrocyanic-acid gas solution were used, yet an equal amount of hydrocyanic-acid gas was recovered from each.

The absence of chemical reaction or of adsorption on the soil particles in pure sand is shown in experiments 4 and 4a. Here the gas remains in the tube for five days without any decrease in the total amount present. The difference between a wet and dry soil is again shown in experiments 4 and 5.

TABLE X.—*Summary of experiments with a soil tube*

Experiment No.	Kind of soil.	Quantity of air aspirated through tube.	Interval before testing.	Moisture.	Amount of air required to cause turbidity with silver nitrate.					
					1st section.	2d section.	3d section.	4th section.	5th section.	6th section.
		Liters.	*Hours.*		*C.c.*	*C.c.*	*C.c.*	*C.c.*	*C.c.*	*C.c.*
1.....	Clay.....	108	.....	Moist.....	120	120	120	135	145	160
1a.....	...do.....	108	48	...do.....	330	240	420	450	820	1,200
2.....	...do.....	30	.....	Wet.....	120	130	145	1,080	.....	.....
3.....	Sand....	66	.....	Moist.....	105	105	105	140	140	210
3a.....	...do.....	66	16	...do.....	(a)	.....	.....	.....	.....	.....
4.....	...do.....	30	.....	Dry.....	75	75	130	130	220	500
4a.....	...do.....	30	120	...do.....	200	170	210	200	190	180
5.....	...do.....	30	.....	Wet.....	390	380	380	310	310	300

a No trace HCN.

SUMMARY

(1) The toxicity of hydrocyanic-acid-gas solutions varied with the insects experimented upon, from the minimum for houseflies of 0.0156 gm. of sodium cyanid per liter (equivalent to 0.0046 per cent of hydrocyanic-acid gas over the solution) to the maximum for beetles of 0.125 gm. sodium cyanid per liter (equivalent to 0.0365 per cent of hydrocyanic-acid gas over the solution), the latter being the most resistant of any insect experimented upon.

(2) Gas from a solution of approximately the same strength as that used above on houseflies retarded the germination total of lettuce seed 11.3 per cent.

(3) Lettuce seed is not killed by two days' exposure to hydrocyanic-acid gas as strong as 0.0366 gm. of sodium cyanid per liter (equivalent to 0.0109 per cent of hydrocyanic acid gas over the solution) and will give a good germination percentage if removed at the end of this time.

(4) Stimulation was greatest at a point one sixteenth of that causing retardation—viz, 0.0011 gm. of sodium cyanid per liter (approximately 0.00033 per cent of hydrocyanic-acid gas over the solution).

(5) Solutions of hydrocyanic-acid gas approximately 256 times as strong as that necessary to produce gas having the minimum killing strength for flies were fatal to all seedlings tested and to 50 per cent of the cuttings placed in the solution, while a solution approximately twice as strong as that required to produce a gas concentration fatal to flies had no effect even upon seedlings.

(6) Sodium-cyanid solutions introduced into the soil failed to give a trace of hydrocyanic-acid gas in air drawn from the soil.

(7) The use of pressure in forcing gas into the soil did not materially increase the rate at which it could be introduced.

(8) Soil and water are both strong absorbents of hydrocyanic-acid gas. Retention of hydrocyanic-acid gas by the soil is dependent upon the character of the soil, while that of water remains constant under uniform conditions of pressure and temperature.

(9) The variability of gas absorption by the soil makes it practically impossible in field work to estimate the dosage of sodium cyanid required to give a toxic effect on insects and at the same time to be within the margin of safety to plants.

(10) In small amounts of soil of a uniform character it is possible to determine experimentally the margin of safety between certain insects and plants.

(11) A heavy damp or a very wet sandy soil is almost impervious to hydrocyanic-acid gas.

(12) A pure sandy soil when wet will take up hydrocyanic-acid gas only in proportion to the amount of water present and this may again be given off, but gas in contact with a clay soil either enters into a chemical combination with some of the soil constituents or is adsorbed by the soil particles.

(13) Gas generated in a soil body diffuses with extreme slowness in clay soils or very wet sandy soils, but in sand with a medium amount of moisture, the diffusion of gas is much more rapid.

(14) The use of sodium cyanid offers a satisfactory means of fumigating masses of loose, porous soil, especially those with only small amounts of clay, or of seed beds and potting soil. Such treatments allow of much wider range of concentrations when the soil is not occupied by a crop.

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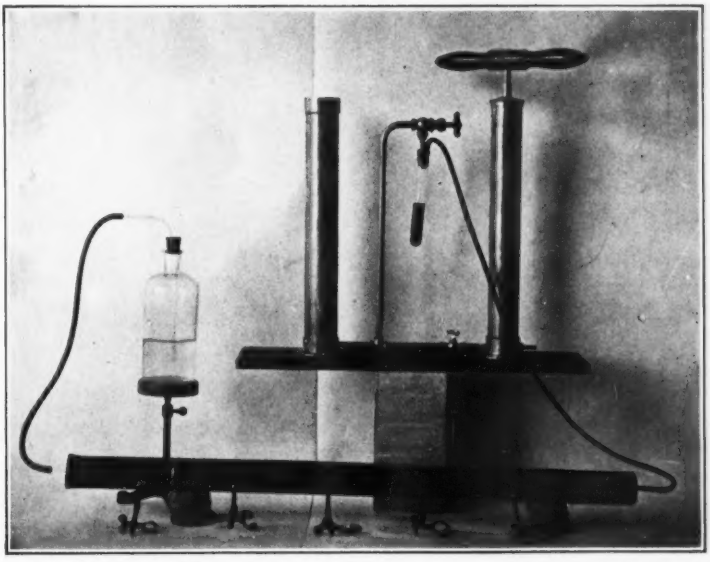
PLATE 40

Soil tube connected to air pump and jar containing hydrocyanic-acid-gas solution.



ENZYMS OF MILK AND BUTTER[1]

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INTRODUCTION

The deterioration of butter during storage is often attributed to the enzyms in the buttermilk which it contains. There has been some experimental study of the matter; but most of the published results of these investigations yield little information concerning the actual enzym content of butter, because of the fact that the studies dealt chiefly with the chemical changes in the butter itself during storage, and sufficient care was not used to prevent possible contamination by microorganisms. In fact, a survey of the literature dealing with the enzyms of milk and its products shows a lamentable lack of clearness on the part of the investigators in distinguishing between the enzyms of milk itself and those which are due to bacterial infections.

Rogers (*16*)[2] found lipase to be the cause of the increase in the acidity of canned butter on standing. Rahn, Brown, and Smith (*15*) stated that

> all (storage) butter investigated showed an increase of "amid nitrogen," i. e., nitrogen not precipitated by copper sulphate, tannic acid, or phosphotungstic acid.

Their methods were shown to be at fault by Rogers, Berg, Potteiger, and Davis (*17*), who could observe

> no evidence of an increase in soluble nitrogen in butter on long standing at 0° F., even when the conditions of manufacture were most favorable to such changes.

They could detect no proteolysis in buttermilk held a long period of time in cold storage to which 18 per cent of sodium chlorid had been added, but active bacterial proteases, pepsin, and trypsin were not completely inhibited in their action by these adverse conditions. Lactose, they concluded, was oxidized only when a trace of iron and peroxid were added.

During the summer of 1915 one of us (Dahlberg), as a part of his duties in the Division of Dairy and Animal Husbandry of the Minnesota Agricultural Experiment Station, prepared several lots of butter under carefully controlled conditions of manufacture and placed them in stor-

[1] Published, with the permission of the Director, as Paper No. 71 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," pp. 448–450.

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age, in order to study the effect of varying methods of manufacture and storage upon the keeping qualities of the butter. From time to time studies of the bacterial development in the stored butter were made. The results of this work have recently been published elsewhere (*25*).

Since the conditions of manufacture and the bacterial development in these butters were known, they afford excellent material for a study of the enzym content of the butter after storage, and such a study was accordingly undertaken.

REVIEW OF PREVIOUS WORK

The following types of enzyms have been reported to be normally present in cow's milk: an amylase (*27*), a lipase (*9*), proteases (*1–5, 7, 22*) a peroxidase (*19*), salolase (*22*), catalase (*12, 18, 20*), reductase (*20*), and a lactose-fermenting enzym (*21*). Vandevelde (*22*), however, concludes that no lipase can be found in fresh milk and that salolase is present in only very slight amounts; while Grimmer (*10*), as a result of his study of the enzyms of the mammary glands, concludes that salolase, peroxidase, and catalase are the only enzyms which are normally secreted with milk. Further, Van de Velde and Landsheere (*23*) found no amylase in milk and criticize Spolverini's observations concerning peroxidase in milk, holding that its presence there is due to bacterial contamination.

These reports indicate something of the confusion which exists with reference to the normal enzyms of milk. The contradictory evidence which has been presented undoubtedly results, in part at least, from the rather crude methods of study of enzym action which were in use at the time when these investigations were in progress. Unfortunately, little attention has been given to these matters during recent years, when more refined methods of study might have given more concordant results.

The question as to whether any enzyms which may be present in milk will be carried down with the butter in churning has not been generally discussed. Kooper (*13*) states that during butter making catalase remains behind in the buttermilk and is therefore not a direct constituent of butter fat. It might be assumed, however, that any buttermilk which remains in the butter would contain the same enzyms that were present in the original milk. In fact, Hesse (*12*), who found small amounts of catalase in butter, calculated his results on the basis of its buttermilk content. Furthermore, since butter fat is undoubtedly an emulsion colloid, it might be assumed to carry with it into the butter, in the form of absorption complexes, large proportions of the enzyms originally present in the milk. But no definite information concerning these matters, other than that cited in the opening paragraphs of this paper, has been published.

EXPERIMENTAL WORK

I. INVESTIGATIONS CONCERNING GALACTASE

The normal proteolytic enzym of milk, which aids in the slow decomposition of milk proteins into peptones, amino acids, and ammonia, was given the name "galactase" by Babcock and Russell (*1–5*). This name is unfortunately not in accord with the present system of nomenclature, and the enzym ought properly to be called "casease" or "lactalbuminase"; but, since the name suggested by Babcock and Russell has come into common use and its significance is properly understood, we decided to continue its use.

Before undertaking the proposed studies of the galactase content of milk, cream, skim milk, buttermilk, and butter it was necessary to determine upon certain details of technic which had not been satisfactorily worked out by former investigators. This preliminary work may be briefly summarized as follows:

(*a*) LENGTH OF PERIOD OF ACTION.—Previous experimental data give quantitative measures of proteolytic activity for periods varying from 1 hour for certain pepsin studies (*8*) up to the practically prohibitive time of nearly 3 years for galactase (*14*). The time in which most investigators have allowed galactase to act, in order to obtain comparative results, has varied from 30 days to 1 year. This appears to be unnecessarily long. The analytical data presented by Babcock and Russell give an average increase in the soluble-nitrogen content of a number of samples of skim milk, when expressed as percentage of the total nitrogen, of 13.49 per cent for the first 8 days and only 5.66 per cent for the following 12 days. One sample of skim milk increased 26 per cent in soluble nitrogen during the first 7 days, 2 per cent the following 7 days, and to increase the soluble nitrogen content another 26 per cent required a period of 3 days. There is very little difference in the comparative galactase content of various skim milks when analyses made at the end of 7 days or of 6 months are compared. In the present work the period of action was limited to 4 days, since it was found that the soluble nitrogen in skim milk increased 10 or 12 per cent during this time.

(*b*) MEASUREMENT OF HYDROLYSIS BY MEANS OF THE NINHYDRIN REACTION.—The ninhydrin method, as proposed by Harding and MacLean (*11*) for the determination of amino-acid or nitrogen, was compared with the official method of the Association of Official Agricultural Chemists (*26*) for determining the increase in soluble nitrogen in different samples of milk due to protease action (Table I). In the ninhydrin determination the sample was diluted 1 to 10, and 1 c. c. of this used in each analysis, but the results were calculated to the 10 c. c. basis. Bacterial action was inhibited by 0.5 per cent of chloroform. All samples in all the work reported in this paper were incubated at 40° C. for four days.

TABLE I.—*Results with ninhydrin method of estimating the rate of hydrolysis of the proteins of milk*

Sample.	Total nitrogen.	Initial soluble nitrogen.	Initial ninhydrin nitrogen.	48-hour soluble nitrogen.	48-hour ninhydrin nitrogen.	Increase in soluble nitrogen.	Increase in ninhydrin nitrogen.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Boiled skim milk	0.0586	0.0033	0.0029	0.0039	0.0032	1.02	0.51
Skim milk + 3 per cent of sodium chlorid	.0586	.0108	.0029	.0137	.0031	4.95	.34
Skim milk	.0586	.0108	.0029	.0177	.0032	11.77	.51

The ninhydrin method indicated no increase in nitrogen when there was an actual increase of 11.5 per cent in the soluble nitrogen. This was further substantiated by work on a pure casein solution. This gave only a trace of nitrogen by the ninhydrin method, with no increase after evident proteolysis had taken place. This method of estimating the rate of hydrolysis of proteins was therefore abandoned as not being applicable to investigations with milk proteins.

(*c*) THE INFLUENCE OF CHLOROFORM ON PROTEOLYSIS.—To prevent bacterial action in the samples, chloroform was added in amounts found ample by Harding and MacLean (*11*) and Van Slyke (*24*). Skim milk to be analyzed at varying intervals extending over a long period of time was treated with 1 per cent chloroform, which, according to the above investigators and Babcock and Russell (*5*), should have only slightly inhibited proteolysis. As shown in Table II, the soluble nitrogen of this skim milk did not increase on standing. In verifying this result the soluble nitrogen was obtained by a Kjeldahl determination of the nitrogen in the total filtrate from the casein precipitation. Closer agreement in the duplicates could be obtained from the filtrates than from the casein itself.

TABLE II.—*Influence of chloroform on the proteolysis of milk as indicated by soluble nitrogen*

Sample.	Chloroform.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase in soluble nitrogen as per cent of total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
1. Skim milk	0.5	0.0583	0.0129	0.0183	9.26
	1.0	.0583	.0130	.0114	−2.74
2. Skim milk	.5	.0531	.0115	.0170	10.35
	1.0	.0531	.0115	.0120	.94
3. Skim milk	.5	.0406	.0119	.0136	4.18
	1.0	.0406	.0119	.0103	−3.94
4. Skim milk	1.0	.0547	.0106	.0079	−4.93
5. Buttermilk, neutral	.5	.0551	.0125	.0201	13.60
	1.0	.0551	.0125	.0174	9.80
6. Buttermilk, neutral	1.0	.0463	.0064	.0059	−1.08

Sample 5 was buttermilk obtained from a small hand churning and probably contained 1 per cent of butter fat. Since fat destroys the anesthetic property of chloroform by combining with it, the results of No. 5 should not be considered. In every other case 1 per cent of chloroform in skim milk or buttermilk completely inhibited proteolysis.

(*d*) THE INFLUENCE OF SODIUM CHLORID ON PROTEOLYSIS.—The possibility that the sodium chlorid present in ordinary butter might inhibit proteolytic activity led us to make a study of the effect of varying percentages of sodium chlorid added to skim milk upon the rate of production of soluble nitrogen by galactase. After the period of incubation the unchanged casein was precipitated from the milk by means of dilute acetic acid and the soluble nitrogen in the filtrate determined by the Kjeldahl method. The samples were preserved during the incubation by 0.75 per cent chloroform. The following results were obtained (Table III).

TABLE III.—*Influence of sodium chlorid on the proteolysis of milk (with 0.75 per cent of chloroform)*

Sodium chlorid added.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 21 days.	Soluble nitrogen after 72 days.	Increase.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.0 boiled	0.0561	0.0055	0.0051	0.0034	−3.74
.0	.0561	.0121	.0188	.0198	13.72
.5	.0561	.0121	.....	.0171	8.91
1.0	.0561	.0121	.0142	.0131	1.78
15.0	.0534	.0121	.....	.0110	−2.06
20.0	.0528	.0121	.0120	.0103	−3.40

The effect of sodium chlorid is marked; both 15 and 20 per cent, the concentration of the salt brine in butter, stopped all proteolysis. The very slight increase in the soluble nitrogen content of the skim milk containing 1 per cent of the salt is partially due to the excessive chloroform used, as the following tests, in which only 0.5 per cent of the anesthetic was used, will show (Table IV).

TABLE IV.—*Influence of sodium chlorid on the proteolysis of milk (with 0.5 per cent of chloroform)*

Sodium chlorid added.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 5 days.	Increase.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.0 boiled	0.0586	0.0033	0.0034	0.51
.0	.0586	.0108	.0242	22.86
.8	.0586	.0108	.0201	15.87
3.0	.0586	.0108	.0143	5.97

The actual checking of the increase in soluble nitrogen due to the addition of 1 per cent of sodium chlorid was approximately 10 per cent in either case, but 3 per cent of the salt did not entirely prevent proteolysis.

(*e*) OCCURRENCE OF GALACTASE IN MILK DURING PROCESS OF BUTTER MAKING.—In following the protease of the fresh milk to the finished butter, the reaction of the various products was in every case brought to that of the fresh milk—that is, 10 c. c. were exactly neutralized by 1.5 c. c. of *N*/10 alkali when phenolphthalein was used as an indicator, by dipping a stick of sodium hydroxid into the sample. If the sample became too alkaline, it was neutralized by some of the original sample so that dilution was avoided. The various samples were treated with chloroform in the following proportions: The skim milk, buttermilk, and bowl contents, 0.5 per cent; the milk, 2.5 per cent; and the cream, which contained 23 per cent of butter fat, 5 per cent. The "bowl contents" was an emulsion of the slime in the wash water held in the bowl. An equal volume of boiled skim milk was added as a substrate, but the increase in the soluble nitrogen was calculated on the basis of the bowl contents alone. The "cream during ripening" refers to the proteolysis occurring during the 10 hours it was ripened at 85° F. and the 20 hours it was held at 54° previous to churning.

TABLE V.—*Presence and concentration of galactase in milk and butter as indicated by the increase of soluble nitrogen*

Sample.	Total nitrogen.	Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase.	Initial percentage of total nitrogen as casein.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
Skim milk	0.0583	0.0129	0.0183	11.10	77.87
Whole milk	.0544	.0113	.0198	15.62	79.23
Cream	.0403	.0076	.0142	16.37	81.14
Bowl contents	.0189	.0046	.0262	114.28	89.93
Cream during ripening	.0403	.0076	.0081	1.66	.....
Cream after ripening	.0403	.0081	.0139	14.39	.....
Buttermilk	.0551	.0125	.0201	13.60	.....

It appears from these data that in separating milk, galactase is taken out of the skim milk part, slightly increased in the cream, and highly concentrated in the separator slime. While no relationship exists between the increase in soluble nitrogen and the total nitrogen, it is evident that the factors at work during milk separation which increase the percentage of casein in the total nitrogen also increase the galactase content. The cream underwent a slight proteolytic digestion during the ripening process, but the proteolysis after souring and neutralization was less than that of the sweet cream. This indicates that the chief

proteolytic enzym of milk is not of bacterial origin, as Olson (*14*) recently reported.

(*f*) AMOUNT OF GALACTASE CONTAINED IN BUTTER.—The butter used in the experiments represented both good and bad qualities. The "fresh dairy" butter was made from the cream obtained from the milk of the University Farm herd, and was soured spontaneously without pasteurization. The "fresh creamery" butter was made in a cooperative creamery from sweet pasteurized cream ripened by a commercial starter. Both of these butters were of extra-fine quality. The "stored dairy" butter was the same as the "fresh dairy," except that it had been held in cold storage for eight months. The "fresh centralized" butter was made from the sour cream just as it was received by a central creamery. The last two butters mentioned were of poor quality. The "stored centralized" butter was made in the same way as the "fresh centralized," but had been held eight months in cold storage. It was of extremely poor quality.

No effort was made to obtain a pure enzym extract from the butter. A known weight, usually 400 gm., of butter was melted at 45° C. in two long glass tubes about an inch in diameter. After the separation was complete the clear fat was hardened by immersing the tubes in cold water, and the curd solution was then washed out. If an excess of fat remained in the extract, it was removed by rewarming and centrifuging. This extract was then dialyzed at a temperature never exceeding 13°C., in a parchment dialyzer, until free of sodium chlorid, the last six or eight hours of dialysis being with distilled water. This curd was made up to a given volume and used at once in the various tests.

The acetone-ether method of obtaining a fat-free, dry powder was also tried, but so high a percentage of sodium chlorid was left in the powder that dialysis was necessary. Consequently this method had no advantages over the other, and the enzyms probably would have been weakened by the precipitation.

The casein was precipitated by the optional official method (*26*, p. 118), rather than the official method because filtering was often more rapid, the volume to be filtered much less, and a clear filtrate more easily obtained. (Thirteen analyses of chloroformed-skim milk and butter-curd extracts gave an average of 0.00104 gm. more nitrogen in the form of casein by the use of alum as the precipitant; hence, the methods should not be used interchangeably.) A clear filtrate was more easily obtained and an excess of substrate assured by the addition of boiled skim milk to the curd solutions. In a few cases the filtering had to be done on a "Büchner funnel" through three filter papers and repeated 10 to 20 times to obtain a perfectly clear filtrate. Bacterial action was prevented by 1 per cent chloroform instead of 0.5 per cent because the extracts contained considerable fat (Table VI).

TABLE VI.—*Presence of galactase in butter, as indicated by the increase in soluble nitrogen*

Kind of butter.	Concentration.		Initial soluble nitrogen.	Soluble nitrogen after 4 days.	Increase.	Increase for 10 gm. of butter.
	Ratio, butter to curd extract.	Ratio, curd extract to boiled skim milk added.				
			Gm.	*Gm.*	*Gm.*	*Gm.*
1. Fresh dairy	2:1	50:100	0.0098	0.0108	0.0010	0.0015
Fresh dairy, boiled	2:1	50:100	.0091	.0091	.0000	.0000
2. Fresh dairy	2:1	50:100	.0080	.0090	.0000	.0015
Fresh dairy, boiled	2:1	50:100	.0070	.0045	.0025	.0000
3. Stored dairy	3:2	50:50	.0043	.0063	.0020	.0027
4. Stored centralized	2:1	50:50	.0042	.0078	.0036	.0036
Stored centralized boiled	2:1	50:50	.0039	.0036	.0003	.0000
5. Fresh creamery	3:2	50:50	.0049	.0044	.0005	.0000

The two samples of fresh dairy butter gave uniform and measurable proteolytic action, while one sample of fresh pasteurized creamery butter showed no digestion of the casein. The increase in the soluble nitrogen of the stored butter was nearly twice that of the fresh butter.

II. LIPASE CONTENT OF BUTTER

Lipase hydrolyzes fats into alcohols and fatty acids, causing an increase in the acidity of the media acted upon. The increase in the acidity of butter on standing is supposed to be due to this enzym (*16*).

The curd solutions used in testing for lipase activity were just half the volume of the butter. The substrate was either 5 per cent of butter fat or olive oil, and the preservative was 2.5 per cent of chloroform. Ten c. c. aliquots of the extracts in 10 c. c. of neutral 95 per cent alcohol were titrated against *N*/40 sodium hydroxid, with phenolphthalein as indicator.

TABLE VII.—*Presence of lipase in butter as shown by the increase of acidity*

Kind of butter.	Initial acidity.	Acidity after 4 days.	Increase over boiled.
	Per cent.	*Per cent.*	*Per cent.*
1. Fresh dairy, boiled	0.2	1.3	0.0
Fresh dairy	.2	1.1	.0
2. Fresh unsalted dairy, boiled	5.3	5.3	.0
Fresh unsalted dairy	6.3	6.6	.3
3. Stored unsalted dairy	5.5	5.5	.0
4. Fresh centralized	2.7	3.0	.3
5. Stored centralized	2.4	3.5	1.1
6. Fresh creamery	1.8	2.2	.4

The extracts from the unsalted butters were not dialyzed. This accounts for their higher initial acidity.

Lipase activity during four days at 40° C. was too small to be accurately measured, except in the extract from the stored centralized butter.

III. OXIDASE CONTENT OF MILK AND BUTTER

Since the investigations of oxidase activity in milk have been limited to a few easily oxidized substances and since oxidases show a specificity toward chemicals in their action, it was thought desirable to investigate by Bunzel's method the action of milk on several of the common chromogens (7). Four c. c. of milk were used in each test. Negative results were obtained with paraphenylene diamine, phenolphthalein, hydroquinon, pyrocatechin, phloroglucin, α-naphthol, and para-, meta-, and ortho-cresols. Positive results were obtained with metol and pyrogallol; but since the action of the latter was about 20 per cent the greater, pyrogallol alone was used for the final determinations. The results given in Table VIII were obtained in duplicate, the duplicates agreeing very closely.

TABLE VIII.—*Estimation of oxidase in milk as shown by the oxygen absorption of pyrogallol*

[Readings are expressed as millimeters of mercury, each millimeter representing an absorption of 0.025 c. c. of oxygen.]

Time.	Skim milk 1.	Whole milk.		Skim milk 2.	
		Raw.	Boiled.	Raw.	Boiled.
Minutes.					
60		0.16	0.00	0.00	0.00
80		.40	.00	.00	.00
100		.56	.00	.17	.00
120	0.95	.70	.16	.17	.00
140	1.07	.85	.20	.35	.00
160	1.28	.97	.27	.40	.00
180	1.33	1.13	.40	.....	.....
300	.....	1.70	.91	.....	.....
360	.....	1.97	1.22	.....	.....
420	.....	2.25	1.48	.....	.....
460	.....	2.32	1.55	.....	.....

There was no agreement in the results obtained from the skim milk from the same herd on different days; boiling failed to completely inhibit the activity of the whole milk, and the action was very slow in starting. It can not be caused by a normal milk oxidase. In every case a browning of the samples was quite marked before oxygen absorption started.

Seven curd solutions from different butters gave no action with pyrogallol.

IV. CATALASE CONTENT OF MILK AND BUTTER

Storch's method of measuring the catalase of milk, as given by Barthel (*6*), gave very closely agreeing duplicate results and was used in this work (Table IX). In milk the liberation of oxygen ceased in four hours. The curd extracts were equal in volume to the original butter. Five per cent of commercial hydrogen peroxid was added to the samples to be tested, and the concentration of peroxid in the boiled samples at the end of four hours were used as the basis for calculation.

TABLE IX.—*Catalase content of milk and butter as shown by reaction with hydrogen peroxid*

Sample.	Hydrogen peroxid content after 4 hours.		Reduction of hydrogen peroxid in unboiled milk or butter.
	Boiled.	Unboiled.	
	Gm.	*Gm.*	*Gm.*
1. Boiled milk	0.0054	.....	.....
Milk	.....	0.0039	0.0015
2. Boiled fresh dairy butter	.0054	.....	.....
Fresh dairy butter	.....	.0049	.0005
3. Stored dairy butter	.....	.0049	.0005
4. Fresh centralized butter	.....	.0026	.0028
5. Stored centralized butter	.....	.0039	.0015
6. Fresh pasteurized creamery butter	.....	.0048	.0006

The fresh centralized butter did not contain as much catalase as the results show, because a clear filtrate was not obtained. Every sample of butter contained catalase. That the centralized butter gave higher results than the other butter and as high as milk itself agrees with the belief that there is some relationship existing between catalase activity in milk and bacterial growth. Storage did not diminish its activity.

V. PEROXIDASE CONTENT OF MILK AND BUTTER

The common Storch test for heated milk was used on the butter extracts with the following results:

TABLE X.—*Color changes showing the peroxidase content of milk and butter*

Sample.	Time of heating (in minutes).				
	0	5	10	20	30
1. Fresh dairy milk boiled.	White...	White...	White.....	White.....	White.
Fresh dairy milk.....	...do....	Gray...	Gray.....	Gray.....	Gray.
2. Stored dairy milk.....	...do....	White...	White.....	Faint gray.	Faint gray.
3. Fresh centralized.....	...do....	Gray...	Gray.....	Gray.....	Gray.
4. Stored centralized.....	...do....	White...	Faint gray.	Faint gray.	Faint gray.
5. Fresh pasteurized creamery.	...do....	...do....	White.....	...do.....	Do.

All the samples gave some color change, but for the storage butters and the pasteurized creamery butter it was very slight. Milk diluted 1 to 160 with distilled water and to which 16 per cent of boiled milk was added gave a gray color similar to that of the fresh dairy butter; hence the peroxidase content of butter is very small.

COMPARISON OF MILK AND BUTTER ENZYMS ON THE BASIS OF TOTAL NITROGEN

The fat in butter acts as so great a diluent for the water-soluble constituents that a direct comparison of the enzyms of milk and butter is misleading. The more logical comparison is on the basis of the total nitrogen, since the proteins and enzyms exist in the same colloidal state and ought to be carried into the butter in the same proportions as they exist in the cream. The total protein of the fresh dairy butter was 0.55 per cent, that of the milk from which it was made 3.47 per cent, so the enzymic activities in the butter were multiplied by 6.31. On taking the milk as the standard and its enzymic content as 1, butter was found to contain the following amounts of the various enzyms which were studied:

Galactase	1.100	Catalase	2.000
Oxidase	0.000	Peroxidase	0.008

The galactase content of the butter was approximately equal to that of the milk, the catalase content was double, but the peroxidase content was very much less, and no oxidase activity could be detected.

RELATION OF ENZYMS IN BUTTER TO DETERIORATION DURING STORAGE

As mentioned in the introductory paragraphs of this paper, deterioration in quality of butter during storage has been considered by some investigators to be due to the action of enzyms contained in it. Fat-splitting (lipase) or protein-hydrolyzing ("galactase" or casease) enzyms have been suggested as possible agents in causing deterioration. Our work leads us to conclude that lipases are present in butter in very small amounts, if at all, and that they could not be conceived to be sufficiently active at the low temperature used in butter storage to cause any appreciable change in the quality of the butter. The protein-hydrolyzing enzym we found to be completely inhibited by sodium chlorid in the concentrations which are present in the water contained in all normally salted butters. This fact, together with the known inhibiting effect of low temperatures upon proteolysis by enzyms makes it impossible that the hydrolysis of proteins in the butter by enzyms plays any part in deterioration changes. We conclude, therefore, that enzyms are not to be considered as a factor in the deterioration of butter in cold storage.

SUMMARY

(1) Proteolysis in skim milk was completely inhibited by 1 per cent of chloroform and by 15 per cent of sodium chlorid. Galactase can not act in normal butter because of the high salt content.

(2) In the separation of milk the factors which increase the percentage of casein in the total nitrogen also increased the galactase content. The ripening of cream did not increase the rate of proteolysis.

(3) No oxidase was found in milk or butter.

(4) Only one sample of butter gave any evidence of lipase at the end of four days at 40° C.

(5) The enzym content of butter is very small, because of the high dilution in fat. Expressed on the basis of total nitrogen the butter examined contained as much galactase as fresh whole milk, twice as much catalase, but only one one hundred and sixtieth as much peroxidase.

(6) The cold storage of butter weakens the peroxidases, but has little effect on the catalase and galactase.

(7) Enzyms are present in butter in such small amounts and under such unfavorable conditions for enzym activity during cold storage that they need not be considered as a factor in the deterioration of butter during storage.

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